IX. *Further Observations on Welwitschia.*

By H. H. W. Pearson, *Sc.D., F.L.S.*

Communicated by A. C. Seward, *F.R.S.*

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[Plates 22—30.]

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By the courtesy of His Excellency Herr von Lindequist and the Government of German South-West Africa, a second expedition to Damaraland was made in the summer of 1906–7. Welwitschia was found in flower at Welwitsch and in the neighbourhood of Haikamchab. The material which is the subject of this investigation was collected in these localities in January and February, 1907.*

The cost of the journey was defrayed by a grant from the British Association.

* Pearson, 1907 (*a*) (*b*); 1908. (See Bibliography at end of this paper.)

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I am deeply indebted to Prof. A. C. SEWARD, F.R.S., for his advice on certain points discussed in the following pages, and for his kind assistance in connection with the publication of this paper during my absence from England; to Miss STEPHENS, of Newnham College, Cambridge, for reading the proof; and to other friends who have enabled me to overcome some of the serious difficulties occasioned by the inaccessibility of much of the recent literature dealing with this branch of investigation.

Material was fixed at various hours with a view to obtaining indications as to the effects of large differences of temperature and of atmospheric humidity upon the activities of the cell. The best preparations, containing most of the important nuclear divisions, have been made from material fixed between 4.30 and 7.30 P.M. Speaking generally, the absence of contraction and the precision of the reactions to stains are less marked in ovules collected at other times of the day. At the same time, nuclear division is far from being limited to the latter part of the day, and some good examples (*e.g.*, figs. 77, 78) were obtained between 1 and 2 P.M., when the air temperature is near its maximum.* In the early morning (6 A.M.) and late at night (9–11 P.M.) nuclear divisions are comparatively rare. Only three preparations showing embryo-sacs with the free nuclei in simultaneous division have been obtained. One of these is from a cone fixed in the field at Welwitsch between 1 and 2 P.M. It is to be regretted that all information as to the time of gathering the ovules which yielded the two cases figured (figs. 27, 28) is unaccountably lacking. These were fixed three hours after collection. The preparation showing five of eight nuclei in mitosis in a later condition of the embryo-sac (fig. 43) was obtained from an ovule collected at 6.30 P.M., and fixed one hour later.

A. *The Inflorescence and Flowers* (Plate 22, fig. 1).

The unit of inflorescence, both male and female, is a dense spike or, as it is usually termed, cone. The great profusion with which the cones are frequently produced is very remarkable.† In both cases they are normally borne in compound peduncled dichasia, usually more or less modified by arrest and sometimes by the development of more than two branches at a node; in some cases the arrest is so nearly complete that the inflorescence is represented by a single sessile or shortly peduncled cone. The female inflorescence does not usually bear more than 23 cones which are sessile or nearly so; in the male the branching is usually carried farther and the number of cones is proportionally greater; these are sessile or peduncled (figs. 8A, 8B, Plate 23).

The occurrence of subfoliar inflorescences is far more common than was formerly supposed,‡ and in plants large and small which in other respects appear to be

* PEARSON, 1907 (*b*).

† PEARSON, 1907 (*a*).

‡ HOOKER, 1863, p. 20; PEARSON, 1906, p. 272.

perfectly normal. In some cases successive fertile ridges are formed under the leaf in acropetal order and with almost as much regularity as the axillary ridges in normal plants. When this happens the subfoliar inflorescences are commonly more abundant than those in the ordinary position and, if not confined to one side of the plant, they are never equally developed on both. As a result of this abnormal lateral growth the crown with its leaves is displaced and may turn vertically through an angle as great as 90°. These inflorescences do not arise irregularly from the older part of the stock as in some woody angiosperms, but the abnormality is due rather to the appearance of a floriferous meristematic zone below the leaf as well as, or instead of, in its axil. There is no indication that this is in any way directly influenced by external conditions.

One isolated valley near Haikamchab contained 106 plants, of which 24 had not yet flowered. The youngest of these was certainly older than the seedling figured in HOOKER's Monograph (Plate 2, fig. 1). This was the smallest found, and, judging by such standards as are available, could not be less than 10 years old; it bore a single arrested cone of doubtful sex. Several plants not much more advanced were in flower, and it seems probable from a study of these that the male flowers earlier than the female. This was the only locality visited in which the male plants were to any marked extent more numerous than the female.

The female cone has, at the base, a pair of bracts distinguished from those above them by their acute apices (fig. 8B). Frequently a considerable length of axis separates them from the rest of the cone. Exceptionally the one or two succeeding pairs are of like character. These are either barren or bear in their axils arrested or normal cones,* or cone-bearing branches. The bracts above these become successively broader and have a rounded or very slightly acute apex (fig. 8B). The lower of these—usually about 20, frequently as few as 12—are barren and smaller than those above them. All the upper bracts are fertile. Sixty flowers have been found in a single cone and this number is probably but rarely exceeded. All stages of development may be present in the same cone. Ovules situated around the apex become arrested before they reach the stage at which pollination occurs. These with their subtending leaves form a withered tuft crowning the otherwise naked axis after the fall of the seeding flowers and their bracts.†

As in the female, a pair of acute bracts stand at the base of the male cone and subtend perfect or arrested cones or cone-bearing branches. The next pair or two pairs of bracts are barren; all those above them have flowers in their axils. The number of flowers may be as great as 70, and is usually about 50. A few at the top of the cone do not reach maturity.

Macrospores and embryo-sacs up to a stage containing about 64 free nuclei are

* PEARSON, 1906, fig. 1B.

† HOOKER, 1863, Plate 8, fig. 1.

commonly present in the pith of the axis of the female cone (fig. 1). They are absent from some, but occur in most of those examined. Well-fixed material of the arrested lateral cone-axes is not available; in some of these they have been almost certainly identified; from others they seem to be absent. Hitherto they have not been found in the axis of the male cone.

The macrospore-mother-cell has not been recognised in this position; the macrospore itself occurs among the undifferentiated tissue of the apex. A bi-nucleate sac is shown in fig. 1A. The more advanced stages are found at depths below the apex more or less proportional to their ages. The oldest seen is represented in fig. 1C; save for its elongated form it is quite similar to the corresponding stage in the ovule. It is hardly possible that a sac in such a position can ever be functional.

The occurrence of macrospores and their germination among the sterile tissue of the cone-axis is not the least puzzling of the many remarkable features presented by the life-history of Welwitschia. The morphological nature of the ovule in general is still in doubt and the evidence bearing upon the question inadequate and conflicting.* The view, formerly prevalent, that it belongs to the category of the caulome, is in fact a bud, has fallen into disrepute, and authorities are now for the most part divided in supporting two alternate hypotheses, the one that the ovule with its integuments is a structure *sui generis*, and the other that, whatever its position, it is part of the sporophyll, *i.e.* foliolar. For Welwitschia there is no escape from the conclusion that it is a bud. It is impossible to suppose that in a member of so highly organised a group as the Spermaphyta, spores can be produced indifferently in the ovule and in a vegetative axis. Therefore the cone whose axis contains spores can only be a modified ovule; the axis, a nucellus which has undergone a process akin to proliferation. The nucellus is therefore an axial structure and the ovule with its integuments is a bud. Its axial position and the acropetal order in which its envelopes appear are in accord with this view, which has been adopted by most authors for each of the three genera of the Gnetales.† Lignier,‡ on the other hand, following Van Tieghem, regards the functional ovule in all three genera as foliar in position. The male spike is regarded as an axis bearing bracts and simple axillary flowers. It is implied that the reduced ovule which terminates the lateral axis in Welwitschia is an axial structure, which is in accordance with the view usually held. The male flowers of Gnetum and Ephedra are homologous, but more reduced. But there is introduced into the interpretation of the female spike a high degree of complexity. What is ordinarily termed the female flower is really an inflorescence bearing a single flower reduced to an ovule

* Worsdell, 1904.

† Macnab, 1873; Bertrand, 1878; Strasburger, 1879; Beccari, *ex* Lotsy, 1899; Jaccard, 1894; Lotsy, 1899.

‡ Lignier, 1903.

with one integument inserted on an ovuliferous scale of which no traces remain. This view appears to be based entirely upon analogy with the Coniferæ. It is not supported by the anatomical structure and development of the female spikes of these genera as interpreted by other investigators.

There are indications that the compound inflorescence in Gnetum is derived from a simple spike. As in Welwitschia, there is at the base of the female spike a pair of opposite bracts.* These usually subtend a pair of axillary spikes; † the buds which normally give rise to the latter may, however, remain dormant; * sometimes they develop into normal flowers.‡ It appears, therefore, that in Gnetum the lateral spike is equivalent in origin and position to a flower. In Welwitschia the evidence is less complete. Attention has already been directed to the two acute bracts at the base of the cone and to the varying degrees of development of their axillary buds which, however, so far as is known, never produce normal flowers. It is suggested that the dichasium is derived from the cone by proliferation, and that the lateral cones directly replace flowers, as is known to be the case in Gnetum. According to this hypothesis the axes of the lateral cones are nucelli of theoretically indefinite growth, and the presence and acropetal arrangement of embryo-sacs within them is to this extent explicable. It is unfortunately impossible at present to determine whether the terminal cone of the Welwitschia dichasium ever contains these extra-floral macrospores. They have been found to be absent in some cases, but in the material available the terminal cones cannot be distinguished. But they occur in so large a proportion of those examined, and the terminal cones are so few compared with the lateral, that even if they are present in the former they cannot be confined to them. It is to be expected that they will be found also in the lateral cones of Gnetum. Their absence from the axis of the male cone, if confirmed, is not remarkable on this hypothesis, for the ovule of the male flower apparently never produces sporogenous cells.

The morphology of the male and female flowers of Welwitschia has been much discussed and very diverse conclusions have been arrived at by different authors. There are, in the male flower, a well-marked androecium and an arrested ovular body which all admit to be the homologue of the functional nucellus of the female. But the ovular envelope and the two lower whorls of appendages of the male and the two envelopes of the female have been variously interpreted. The views of several authors are given in Table I, in which the numbers indicate the whorls of appendages§ from below upwards.

These divergent views are the outcome of attempts to derive the one flower from the other or both from a hermaphrodite, assumed to be more or less represented

* Engler, 1908, p. 520, figs. D, F, K.

† Eichler, 1887, fig. 76.

‡ Lotsy, 1899, p. 85, Plate 2, fig. 6.

§ See Pearson, 1906, text-figs. 1 and 2.

Table I.

		Hooker, 1863; Eichler, 1889; Warming, 1892; Engler, 1907.	Parlatore, 1869.	Strasburger, 1872.	Macnab, 1873.	Bertrand, 1878.	Bentham and Hooker, 1880.
Appendages of	Male Flower.	1. Perianth	Bracteoles	Perianth	Perianth	Perianth	Bracteoles
		2. Perianth	Bracteoles	Perianth	Perianth	Perianth	Perianth
		3. Androœcium	Androœcium	Androœcium	Androœcium	Androœcium	Androœcium
		4. Integument	Carpels	Carpels	Carpels	Foliar integument	Integument or imperfect ovary
	Female Flower.	1. Perianth	Bracteoles	Carpels	Perianth*	Androœcium	Perianth ?
		2. Integument	Carpels	Integument	Integument	Integument	Integument

* In a postscript, Macnab adopts Strasburger's view that the outer envelope of the female flower represents two carpels rather than a perianth.

by the existing male flower. Wieland's elucidation of the structure of the amphisporangiate strobilus of Bennettites suggests another basis of comparison.† This shows‡ in longitudinal section an axis (receptacle) on which are arranged, from below upwards: (1) bracts which envelop the whole inflorescence; (2) a hypogynous disc of microsporophylls (caducous, perhaps entirely aborted in some species§); (3) several barren "interseminal" scales whose ends, closely imbricated from below upwards, form the surface of the lower two-thirds of the "ovulate cone"; (4) interseminal scales, and, standing in positions which may be regarded as axillary to many of these, (5) long seed-bearing pedicels. Authorities are not in accord as to the morphological character of these seed-bearing pedicels. Their internal structure is exceedingly like that of the interseminal scales and, mainly on this account, Wieland, whose views carry great weight, regards them as homologous organs—the one fertile, the other sterile sporophylls. On the other hand, Solms-Laubach‖ inclined to the view that the interseminal scales are bracts subtending shoots, each of which bears a single terminal ovule. Scott¶ admits the possibility of this view, and, like Solms-Laubach, suggests an analogy with the paleæ and florets

† Such a comparison is briefly but unfavourably considered by Wieland (1906, p. 230).

‡ Wieland, 1906, p. 110, figs. 56, 87, 88.

§ Wieland, *loc. cit.*, pp. 130, 131.

‖ Solms-Laubach, 1891, p. 448.

¶ Scott, 1900, p. 476; see also Coulter and Chamberlain, 1901, p. 144.

respectively on the receptacle of many Compositæ. While most authors agree with WIELAND* in regarding the seed-pedicels as foliar structures, the point is not definitely settled, and it may therefore be permissible to compare the ovulate strobilus of Bennettites with the female cone of Welwitschia.

The Welwitschia cone (♀) shows an elongated axis whose vegetative apex remains active to the last. Upon it are borne from below upwards (see Table II): (1) one (sometimes two or three) pairs of acute bracts; (3) about 10 pairs of imbricating barren bracts increasing in size from below upwards (see fig. 8B); (4) bracts, similar to (3) but larger, bearing in their axils sessile or very shortly stalked female "flowers." This differs from the ovulate strobilus of Bennettites in that (1) the hypogynous disc of microsporophylls is absent; (2) the bracts are in whorls of two instead of being arranged spirally; (3) the upper part of the cone bears only one bract to each flower, instead of five or six. The comparison may be extended to the ovules. In both cases the ovule is orthotropous and surrounded by a single integument which is prolonged upwards into a micropylar tube, the only ovular structure to project beyond the bract. Outside the integument in Bennettites is a "cup-shaped supporting basal husk" formed of "stringy cortical cells several cells deep at the base and thinning out towards the tip."† A characteristic feature of the so-called "perianth" of Welwitschia is the great development in the lateral wing-like extensions of its wall of tubular cells, long, tortuous, and "stringy."‡ It is, however, a much more highly organised structure than the cupule of Bennettites, for, in addition to its form, which indicates an adaptation to the purpose of seed-distribution, it contains a vascular tissue. There are also internal characters which possibly indicate an even more striking resemblance between these ovules, and which will be referred to in a later page (see p. 386).

As to the male flower, WIELAND remarks,§ "nor is there from a plain point of view an unbridgeable gap between the staminate disc of Cycadeoidea and that of Welwitschia, for the latter could arise similarly to one of the hypothetical one-seeded and bisporangiate forms of the Cycadeoidean alliance by one of the simplest of all evolutionary processes, namely, increased number of flowers to the plant and decrease in bulk until there was left of each original frond but a single filament bearing . . . pollen sacs." The cone itself might be derived from the flower by a process of proliferation.‖

It appears, therefore, that if the ovule of Bennettites is axial it is theoretically possible to derive both the female cone and the male flower of Welwitschia from an amphisporangiate strobilus of this type. The former requires the abortion of the

* *Cf.* ARBER and PARKIN, 1907, pp. 55 and *sqq.*

† WIELAND, pp. 120, 121.

‡ HOOKER, 1863, Plates 7 and 8; STRASBURGER, 1872, Taf. 18, fig. 22.

§ WIELAND, 1906, p. 245.

‖ WIELAND, 1908.

disc of microsporophylls—a tendency towards which is perhaps shown by the fossil forms themselves—and minor changes in the relative growth of the main axis and of the lateral members, and in the number of the sterile appendages as compared with the fertile lateral axes. The latter demands reduction and specialisation in the microsporophyll-whorl, the disappearance of the interseminal scales, the reduction of the ovulate axes to one terminal arrested ovule and the abortion of one of its envelopes. The suggested relationships of the various parts are expressed in the following tables.

This view seems to account more naturally than any hypothesis yet advanced, not only for the separated sexes, but also for the relations between the parts of the male and female flowers. The ovules in both flowers are clearly cauline and it must be assumed that they have come from a type in which this was also a character. Ancient types, in which the ovule was apparently axial, are known, and, if the comparison with Bennettites is invalid, it may still be that the relations between the Welwitschia cones are of the nature suggested, viz., that the female cone and the male flower are derived by two independent series of reductions from a primitive amphisporangiate strobilus with axile ovules.

Pending a detailed anatomical investigation, the internal structure of the

Table II.

No.	A. Bennettitean amphisporangiate strobilus.*	B. Welwitschia female cone.	C. Welwitschia male flower.†	D. Angiospermic hermaphrodite flower with a single orthotropous cauline ovule.
1	Bracts at base of strobilus .	1–3 pairs of acute bracts at base of cone	Perianth—2 whorls	Perianth—2 whorls; or variously reduced.
2	Hypogynous disc of microsporophylls, caducous, possibly aborted in some forms	Aborted	Androecium . .	Androecium.
3	Barren interseminal scales on lower part of ovulate cone	Barren bracts on lower part of cone	Aborted . . .	Aborted.
4	Interseminal scales, many of which subtend ovulate axes	Bracts, each subtending a sessile axial ovule	Aborted . . .	Closed carpels.
5	Ovules, each with one integument and a basal husk‡	Ovules, each with one integument and a winged "perigone"‡	One terminal arrested ovule with one envelope‡	One axial ovule with two integuments.‡
6	Cone-axis	Cone-axis	Floral axis—arrested	Floral axis—arrested.

* WIELAND, 1906, text-figs. 56, 87, 88.

† PEARSON, 1906, text-fig. 1.

‡ See Table III.

Table III.

Bennettitean ovule* and envelopes.	Welwitschia female† flower.	Welwitschia male‡ flower.	Ovule of the angiosperm.
1. Ovular body	Present	Present (arrested) . . .	Present
2. Integument with elongated micropylar tube (pollen-receiver)	Present; micropylar tube much elongated (pollen-receiver)	. . . . .	Present as one integument
3. Basal husk, an outgrowth from the "pedicel" mainly composed of long tubular cells	Present (?) as the laterally winged outer envelope. The wings are very largely composed of long tubular cells	One of these is aborted .	Probably not represented
	See Table II	4. Androecium 5. Perianth	

* WIELAND, 1906, text-fig. 63.

† PEARSON, 1906, text-fig. 2.

‡ PEARSON, 1906, text-fig. 1.

cone-axes, bracts, and floral appendages§ may be briefly compared. The vascular structure of the peduncle of the female cone has been described by STRASBURGER‖ and by WORSDELL.¶ The axis is occupied by a parenchymatous pith containing a few sclerotic cells and elongated "spicular" elements. In the peduncle of the male cone the bundles of the inner ring border upon an equally massive pith of a different character. The cells, of which it is almost entirely composed, are from six to twelve times as long as they are broad; they are uniformly arranged, with their axes parallel with that of the peduncle. Their walls are lignified and contain very numerous circular or oval simple pits. They are broader and shorter than the tracheids of the xylem, but perhaps represent a more extensive development of the sclerotic cells in the pith of the female peduncle, which WORSDELL, "although with some hesitation," inclined to regard as the representative of centripetal xylem.¶ Mixed with them are a few spicular cells, which are less common than in the corresponding region in the female. The vascular system is more abundantly developed in the female than in the male.

The acute bracts which stand at the base of both cones are also alike in structure, though differing in form. The bundle is endarch and the xylem appears in transverse section as a narrow plate lying along the inner face of the phloem. Radiating from it

§ I am indebted to Mr. H. BOSMAN and Mr. P. DE V. MOLL for the preparation of a series of sections of these structures.

‖ STRASBURGER, 1872, Taf. 20, figs. 49–51.

¶ WORSDELL, 1901.

in all directions, from the phloem as from the xylem, are short lignified cells, irregular in shape, whose walls are reticulately thickened. These are undoubtedly transfusion-cells, though their distribution does not favour the view that they represent centripetal xylem.

The fertile bracts of both cones have the same vascular structure, which differs in some respects from that of the basal bracts just described. The transfusion elements are entirely lacking,* and there is nothing that can be interpreted as centripetal xylem. The protoxylem usually stands at the apex of a horseshoe whose arms are formed by the metaxylem.

In the outer envelope of the female flower the bundles are much reduced. There are no transfusion elements. Both inner and outer whorls of the perianth of the male are without vascular tissue.

There is therefore nothing in the vascular structure of these organs to support the homologies suggested in Table II. On the other hand, the absence of vascular tissue from the extremely reduced members of the perianth of the male flower is not sufficient to prove that they are not homologous with the acute bracts at the base of both male and female cones. The similarity of structure between the fertile bracts of both cones occasions greater difficulty, since it might be expected, on the hypothesis advanced in Table II, that all the bracts of the male cone would show the same vascular structure. The fertile bracts differ from those at the base of the cone, mainly in the absence of transfusion cells. Since these are so differently developed in the peduncles of the two cones, their absence from the fertile bracts of the male is quite possibly of physiological significance only.

In its reduced hermaphrodite flower and the presence of floral envelopes forming a physiological perianth, Welwitschia approaches the condition of the angiosperm more nearly than does any other living gymnosperm. In considering to what extent the floral structures of Welwitschia and the angiosperms are morphologically comparable, we are met at the outset by the question of the nature of the ovule. That it is a bud in Welwitschia is held to be established. It will be seen later that there is important evidence in favour of regarding Welwitschia and the angiosperms as representing at least two distinct lines having their common origin in a group which must have been preceded by archegoniate spermaphytes. It is therefore to be expected that an axial ovule should occur in the more primitive angiosperms, as in Welwitschia. Many examples of ovules occupying an axial position are recorded† among the angiosperms, and these in groups which, on other grounds, have been regarded as primitive—"indeed, there is constantly increasing evidence to show that the single axial ovule is the primitive form."‡ In view of what obtains in Welwitschia, it is difficult to

* STRASBURGER, 1872, Taf. 20, figs. 47, 48.

† STRASBURGER, 1879, pp. 49 *et seq.*; CAMPBELL, 1900, 1901; COULTER and CHAMBERLAIN, 1903, p. 46.

‡ CAMPBELL, 1900, p. 5.

avoid the conclusion that in these forms the ovule is not merely an axially placed sporangium with a distinctive tegumentary system,* but a modified bud. This view, if correct, would seem to point to the conclusion that the ovule throughout the group, whatever its position, is cauline. On the other hand it is, perhaps, not necessarily regarded as in all cases belonging to the same morphological category.† But the point of immediate interest is that in some angiosperms reasonably regarded as relatively primitive the ovule is apparently an axial structure, as in Welwitschia. There is therefore suggested in Tables II and III a comparison between the floral members of such an angiosperm and those of Welwitschia. If the relations which there appear can be maintained, neither of the Welwitschia flowers possesses a structure homologous with the ovary. A possible alternative is that the outer winged envelope of the female flower is a primitive ovary—as has already been suggested by PARLATORE and other authors (see Table I). In this case the ovular axis produces the ovary as a pair of lateral appendages—a view which is very difficult to reconcile with the relations between the ovule and the carpel, which commonly obtain among the angiosperms. It seems, therefore, more probable that if the carpel is represented at all in the ovulate cone of Welwitschia it is by the bract which subtends the ovule. On this hypothesis, if we imagine the cone so much reduced that the arrested axis bears only two bracts and a single ovule in the axil of one of these, we arrive at a structure closely resembling the young flower of Dieffenbachia‡—a reduced inflorescence in the same sense that the female cone of Welwitschia and, as suggested above, the ovulate portion of the Bennettitean strobilus, are inflorescences.

At a later stage the embryo-sac of Peperomia will be compared with that of Welwitschia, and the results will tend to confirm CAMPBELL'S conclusions that it represents a primitive type of angiosperm sac. Many writers have maintained that Peperomia is a reduced form both as regards its vegetative characters and its floral structures.§ While these views are not necessarily incompatible with CAMPBELL'S interpretation of the embryo-sac, the new light which Welwitschia throws upon the latter is rather favourable to the relatively low position usually assigned to the genus by recent taxonomists.‖ To this ARBER and PARKIN have advanced objections.¶ The most important of these are: (1) that, on the assumption of the origin of the flower from a Bennettiteoid strobilus, it presupposes that the perianth arises *de novo*, and (2) that Peperomia and other forms with simple apetalous flowers invariably possess a complicated and highly evolved inflorescence. The first objection raised is undoubtedly serious if the angiosperms are monophyletic, and the flower throughout

* BALFOUR, 1901, p. 822.

† *Cf.* EICHLER, *ex* WORSDELL, 1904.

‡ CAMPBELL, 1900, fig. 3.

§ *Cf.* T. G. HILL, 1906; ARBER and PARKIN, 1907.

‖ ENGLER, 1907; see also A. W. HILL, 1906, p. 425.

¶ ARBER and PARKIN, 1907.

is derived from what the authors term the "Proanthostrobilus." But while so little is known of the life-histories of so many angiospermic families, and the phylogeny of the group is so obscure, evidence tending to show that Peperomia and other apetalous forms are relatively primitive is not invalidated by the presence of a perianth in other groups. On the "proanthostrobilus" hypothesis, it is certainly not easy to account for the dense spike of a primitive Peperomia. But too much importance may easily be attributed to this point. The male cone of Welwitschia—really a dense spike of reduced hermaphrodite flowers—presents no less difficulty, and is very considerably more primitive than either the spike of Peperomia or the flower of a Magnolia.

Physiologically the integument of Welwitschia is to some extent comparable with the ovary of the angiosperm. Not only does it receive the pollen, but it also furnishes some, at least, of the nutrient medium in which its germination and, frequently, the early growth of the pollen-tube takes place (fig. 11). The outer envelope of the fertile ovule performs two of the functions commonly associated with the ovary of the higher plants, viz., the protection of the ovule and the distribution of the seed—for which its winged form is admirably adapted.* In this it is assisted by the broad subtending bract to which the seed remains adherent for a time after the disintegration of the cone.†

B. *The Microspore-mother-cell and the Microspore* (Plate 22, figs. 2–7).

The microsporangium formerly figured‡ contains pollen-mother-cells in the resting condition. The loculus undergoes a further increase in size and the cells of the inner layer of the tapetum (fig. 2, *i.w.*) become considerably larger. At this time they are almost constantly bi-nucleate and their cytoplasm is very scanty. The outer tapetal cells (*m.w.*) are very much flattened and the three wall-layers now resemble those of Ephedra in a somewhat later stage.§ The breaking down of a number of the spore-mother-cells seems to be a normal occurrence: not infrequently the loculus contains nothing but the disorganised remains of the whole cell-mass. The wall of the mother-cell shows no appreciable increase in thickness during its development.

The number of the chromosomes in the heterotypic division lies between 22 and 26, and is believed to be 25. The spindle is at first multipolar, and in one stage closely resembles a condition figured by MOTTIER for Tradescantia.‖ The stout fibres of the bipolar spindle show slight curvatures in opposite directions at the poles (fig. 3). When the daughter-nuclei are organised there is seen to be no cell-plate between them (fig. 4).

* HOOKER, 1863, Plate 7, fig. 3.
† *Loc. cit.*, fig. 2.
‡ PEARSON, 1906, fig. 4.
§ *Cf.* LAND, 1904, fig. 14.
‖ MOTTIER, 1907, fig. 40.

During the homotypic divisions the cytoplasm possesses a greater density than in the preceding mitosis, and this increases up to the organisation of the microspores. The spindles are frequently placed at right angles to one another (fig. 5), but they may be inclined at any angle, and sometimes their axes are parallel. Usually the daughter-nuclei show the tetrad arrangement (fig. 6); sometimes a section passes through them all. In addition to those of the two spindles, fibres appear between the two pairs of daughter-nuclei and separating walls are formed in the usual manner (fig. 7). At the same time a new wall is formed in the periphery of the cytoplasm, and there result four free microspores still enclosed in the wall of the mother-cell.*

The account already given of the structure of the adult pollen-grain is confirmed. But the nuclei are not confined to the positions formerly indicated;† they have frequently been seen together at one end of the cell, and sometimes the generative cell and tube-nucleus are separated by the whole length of the cell. It is therefore possible that in the living pollen-grain they may be situated in any part of the cytoplasm; but the thick spore-wall no doubt prevents rapid fixation, and the cytoplasm is always more or less contracted.

C. *Pollination* (Plate 23, figs. 8A–11).

Pollination is mainly effected by the hemipterous insect *Odontopus sexpunctulatus*.‡ The pollen is received by a drop of a sweet fluid which stands on the top of the projecting micropyle when the ovule is ready for fertilisation. The secretion of this fluid undoubtedly takes place largely in the cells forming the inner lining of the integument and micropyle (figs. 9–11, $ep._2$) and the outer covering of the nucellar cone (figs. 9, 10, and text-fig., $ep._1$, page 365). Fig. 9 represents a longitudinal section of these and neighbouring layers in a young ovule not yet ready for pollination. In fig. 10 is shown a transverse section of the same layers in an ovule which contains young embryos—*i.e.* when the secretion has ceased. The greatly enlarged cells of the layer, $ep._1$, would lead to the conclusion that it plays a predominant part in the function. Both layers, together with the outer cells of the integument, of an ovule at the stage of pollination give a copious reaction for cane-sugar with Fehling's solution.§ The appearance of sugar is accompanied by a depletion of the starch which, in the stage of fig. 9, is contained in several hypodermal layers of the nucellar cells (*cf.* 10). The excretion of the sugar solution only commences after sunrise and ceases very soon after, if not before, sunset.‖ It was not determined how long an

* PEARSON, 1906, fig. 11.

† *Loc. cit.*, fig. 16.

‡ This is in direct contradiction of a statement formerly made in error: see PEARSON, 1907 (*a*).

§ Only dried material is available for this reaction; it is quite possible that other regions of the nucellus, internal to those indicated, are also concerned in the secretion of the micropylar fluid.

‖ PEARSON, 1907 (*a*).

ovule remains in the condition for pollination, but a drop probably appears on the top of the micropyle day after day for two or three days at least.

The ovule frequently receives so large a number of pollen-grains that they fill a considerable length of the micropyle (fig. 11). It is probable from their position and number that they sink in the fluid by reason of their own weight. Germination occurs while the pollen-grain lies on the top of the nucellar cone* or, very frequently, at some distance above this in the micropylar fluid (fig. 11). This is to some extent comparable with the growth of the pollen-tube down the style of an angiosperm, but in Welwitschia the nutrient medium in which it grows is partly derived from the nucellus.

D. *The Macrospore and the Embryo-sac* (Plates 23 and 24, figs. 12–23).

An ovule appears never to contain more than a single macrospore-mother-cell. In spite of prolonged search only one stage of the heterotypic division (fig. 12) has been found. It is considerably later than that formerly figured† and is apparently a stage in which the spireme is recovering from synapsis. This condition has been seen several times and it is probable that other stages are passed through with greater rapidity.‡ The spireme presents a beaded appearance owing to the presence in it of very numerous granules, irregular in form and size, staining faintly in diamant fuchsin. Here and there a longitudinal splitting of the thread is suggested.

The division of the daughter-cells has not been seen. Since three embryo-sacs are occasionally present it may be inferred that at least the lower one undergoes a homotypic division.

In general only one macrospore germinates. Two have been found in a few ovules, most of which were taken from the same cone. In these cases they usually lie one above the other, the lower one, which survives, being more advanced in development; rarely are they situated side by side. In one ovule three macrospores were germinating: two were side by side and equal in development and the third above them less advanced.

The macrospore is usually broader at the base than at the apex (fig. 13), and these relations are commonly maintained up to the time of septation. The wall is thick; it shows no signs of cutinisation. The cytoplasm, in which the large nucleus occupies an approximately central position, contains numerous small vacuoles fairly uniformly distributed through it.

The late prophase of the mitosis of the macrospore-nucleus is shown in two sections, oblique to the axis of the spindle, in fig. 14, A and B. The arms of the spindle are slightly curved in opposite directions at the poles; the fibres are faintly stained in

* Pearson, 1906, fig. 17.

† Pearson, 1906, fig. 19A.

‡ *Cf.* Mottier, 1907, p. 314.

erythrosin. At each pole is a vacuole distinguished from the rest by its definite outline (*v.*). The chromosomes are small; their number is not less than 22 nor more than 26. The daughter-nuclei are formed not far from the poles of the cell (fig. 15); there are no constant visible differences between them in form, size, or staining capacity. They have not been found in division, but in the next stage (fig. 16) the nuclei are always approximately equidistant and the slight polarity of the sac has disappeared. This is a point of some interest, for it has been suggested that in Gnetum the descendants of one of the two nuclei of the stage of fig. 15 (the micropylar) are all sexual in function, while those of the other are sterile*—a view which naturally leads to a comparison with the micropylar and antipodal groups respectively of the angiosperm-sac. The distribution of the nuclei in this early stage (fig. 16) of the Welwitschia sac offers no support to this hypothesis; on the contrary, all their visible characters indicate that there are no fundamental differences, actual or potential, between them. Further evidence in confirmation of this conclusion will become available later.

In the bi-nucleate sac figured (fig. 15) the cytoplasm contains, near the periphery and almost midway between the nuclei, a spherical vacuole with a very definite boundary lined with small irregular granules staining brilliantly in erythrosin. This bears some resemblance to the polar vacuoles of fig. 14 and is probably a structure of the same kind. It is not a constant feature of this condition of the sac, nor has it been observed in a later stage.

Each of the four nuclei in fig. 16 is surrounded by dense cytoplasm, outside which there is a marked development of small vacuoles. The absence of a large central vacuole, which was formerly† stated to be a constant feature of the free-nucleate condition, is confirmed. This can only mean that, in proportion to the increase in the area of the macrospore-wall, the growth of the cytoplasm proceeds more rapidly than is usual among Gymnosperms. Whether this is due to an abnormally rapid formation of new cytoplasm, or to an unusually slow increase in the peripheral area of the sac, or to both, is not certain. The small size of the sac at the time of its septation, together with the fact that the vacuole is similarly absent in Gnetum when the normal growth of the sac is hindered,‡ favours the second view. Other evidence, however, makes it probable that the absence of the vacuole is, in part at least, directly due to the rapid growth of the cytoplasm and multiplication of the nuclei. In a cone, most of whose ovules already contain embryos, a few near the apex are still in early stages of development. In these the free nuclei always show a tendency to confine themselves to a lining layer of cytoplasm and the development of vacuoles is much greater than in corresponding stages in younger cones (fig. 17). This appears to be a natural consequence of the impaired vegetative activity of the old cone in

* Lotsy, 1899, p. 92.

† Pearson, 1906.

‡ Karsten, 1892 p. 212.

which growth is mainly centred in the endosperm and embryos. According to LAWSON,* the presence of a large vacuole is favourable to, if not a determining factor of, the rapid growth of the sac, and its absence might therefore be considered as a sign of slow growth. It is not clear, however, that the large central vacuole is rightly regarded as a cause rather than an effect of rapid growth. The requisite degree of turgidity is as well maintained by cell-sap in a series of small vacuoles as by a large mass of fluid in the centre of the cell; and, with a sufficiently rapid rate of growth, the food materials would be used up so rapidly that their accumulation in the fluids of the cell could not occur.

Two stages with the free nuclei of the sac in mitosis are represented in figs. 18, 19; these are from the same cone. Throughout the cytoplasm are distributed large numbers of irregular granules which stain intensely in diamant fuchsin; these are only seen when the nuclei are actually in division, or appear to have recovered from it only recently. In the latter case, one or more of them frequently lie close to the nuclear membrane, in a position somewhat suggestive of centrosomes. They are presumably proteid granules. There is no information as to the manner of origin of the spindle; the fibres are brought out distinctly by *licht-grün*. Each figure is surrounded by an envelope of cytoplasm of varying thickness, which also stains deeply in *licht-grün*; this is especially marked in all cases at the poles, and calls to mind the "polar cap" which plays a conspicuous part in mitosis in many ferns and seed-bearing plants, including Ephedra.† The simultaneous division of the free nuclei of the embryo-sac, assumed to be of universal occurrence in the Gymnosperms, has been described for *Ephedra helvetica*‡ and for *E. trifurca*.§ JACCARD'S figures are diagrammatic; it appears that in *E. trifurca* the figure is not surrounded by an envelope distinguished by its staining capacity, nor is there any marked accumulation of dense cytoplasm at the poles.

In figs. 20 and 21 are shown two older sacs: the former seems to be the stage immediately following the completion of the mitosis of fig. 19, and is probably the same as that represented in fig. 30 of my former paper‖; the latter, probably corresponding to fig. 31,‖ contains approximately the same number of nuclei as a median section of a later stage (figs. 22 and 23) in which septation has commenced. If, therefore, the mitoses of the free nuclei of the sac are simultaneous up to the stage at which septation begins, the sac of fig. 21 contains the full complement of nuclei. The section is very nearly median, and it shows between 185 and 195 nuclei. Calculated from these figures,¶ the total number of nuclei in the sac is between 1015

* LAWSON, 1907, p. 287.
† DAVIS, 1904, p. 439.
‡ JACCARD, 1894.
§ LAND, 1904.
‖ PEARSON, 1906.
¶ See Appendix I.

and 1098. Assuming the constancy of simultaneous divisions up to this point, the actual number is 1024. In other words, the nuclei present in the sac when septation begins all belong to the 11th generation from the nucleus of the macrospore.

Between these two stages (figs. 20, 21) the vacuoles have become greatly reduced, and the nuclei in the later one are very crowded. Each shows, as in earlier stages, a single nucleolus. Very soon after the attainment of this condition the whole sac undergoes a rapid increase in length. The cytoplasm again becomes vacuolated and the nuclei less crowded (figs. 22, 23), while the sac itself attains a length three times that of fig. 21 (*cf.* outlines A and B, fig. 89). In the upper fourth (fig. 22) the nuclei are less crowded, and the cytoplasm more vacuolated than in the lower three-fourths (fig. 23); it may be inferred that the upper end of the sac increases in length more rapidly than the rest. This is also indicated by the fact that, while the antipodal end still lies free in the products of decomposition of the nucellar cells, the micropylar apex has pushed its way into the midst of nucellar tissue whose continuity remains unbroken. These changes and those which immediately follow them are effected with great rapidity and stages intermediate between those of figs. 21 and 22, 23 have rarely been seen; while they are in progress the whole sac is exceedingly liable to contraction during preparation. As in the sac of fig. 21, so in that of figs. 22, 23, the nuclei themselves are alike in all visible characters; in intermediate stages, those of the apical fourth show a somewhat greater avidity for stains than the rest, but this difference persists only for a short time. The nuclei of figs. 22, 23 are crowded with chromatin granules to such an extent that the nucleoli are obliterated; in some other preparations of similar age a single nucleolus in each is recognised with difficulty.

The sac now shows for the first time a clear differentiation into two regions, the one fertile and the other entirely, or for the most part, sterile. The more widely separated nuclei of the upper fourth (fig. 22) are functionally sexual; of the more crowded ones of the lower three-fourths (fig. 23), all, or the great majority, will presently give rise to a sterile nutritive tissue, the endosperm; a few of the latter however frequently remain functionally sexual and behave exactly like those of the fertile end. The fertile nuclei of the upper end are few compared with those which form the sterile tissue. It follows, therefore, that if the simultaneous divisions are maintained up to this stage—and there is no evidence that they are not—the suggestion referred to above, that the sexual nuclei are all descended from the one and the remainder all from the other of the two daughter-nuclei of the macrospore-nucleus, is completely disproved.

The cytoplasm of the section from which figs. 22 and 23 are taken is already marked out into areas by tortuous lines, which stain deeply in *licht-grün*; these are sections of plates of denser cytoplasm, the boundaries of compartments, into which the sac is subdivided. These initial planes of septation bear no visible relation to the spindles formed in the last division of the free nuclei (*cf.* figs. 21, 22, 23); and since the sac undergoes so considerable an amount of growth, resulting in the wider

distribution of the nuclei between these stages, they are almost certainly independent of nuclear division. But since an enucleate compartment has never been seen, it is probable that their positions are in definite relation to the distribution of the nuclei. Their formation is probably the result of a process of cleavage similar to that described for certain Myxomycetes and Phycomycetes,* and resembling in some respects that which precedes the organisation of the pro-embryonal cells in the egg of Ephedra.† The stage immediately preceding that shown in figs. 22, 23 has not been seen, and it has not been determined whether the formation of these cleavage planes is centripetal or whether they appear simultaneously throughout the sac. Near the periphery the cleavage lines usually end in depressions or deep furrows in the cytoplasmic surface. It is therefore possible that they commence, as in the sporangium of Synchitrium,* in these superficial furrows and advance centripetally. However this may be, the cleavage of the micropylar cytoplasm occurs in exactly the same way as that of the lower part. It appears from fig. 32, in which the cytoplasm is probably contracted, that the cleavage planes of fig. 23 really consist of two cytoplasmic surfaces in contact; here they are slightly separated by a clear space, in which no trace of a cell-wall can yet be detected. Between these surfaces the cell-wall is formed and, in the stage of figs. 25–33, reacts to cellulose stains. The compartments of the micropylar fourth are somewhat larger, and include fewer nuclei and larger vacuoles than those of the lower three-fourths. In the former the nuclei are frequently not more than two, only exceptionally more than six‡ (figs. 22, 24, 25); in the latter their number is variable and, owing to the irregular form of the compartments, not easily determined; it is usually twelve or more, occasionally as small as two. There is no indication that any factor is concerned in bringing about this unequal distribution of the nuclei except the unequal growth in length of the sac after the stage of fig. 21 (see below, p. 354). The nuclei themselves, however, are of the same generation, and are equal in every respect, save only that there may still be retained some traces of the difference in staining capacity referred to above. All the nuclei of the stage of figs. 22 and 23 are potentially sexual.

From this stage onwards the two ends of the sac develop along different lines, and their later histories are most conveniently considered separately.

E. *Micropylar End of the Sac* (Plates 24 and 25, figs. 24–31).

The nuclei of the micropylar compartments increase in size and the single nucleolus, once more distinct, is situated in a large clear space (figs. 24 and 25). Those of the lower part of the sac later undergo the same changes (*cf.* figs. 32

* HARPER, 1899, *ex* MOTTIER, 1904, p. 36.

† LAND, 1907, figs. 6–9.

‡ The statement formerly made (PEARSON, 1906, p. 291), that "until a considerably more advanced stage more than two nuclei are not found" in these compartments, is incorrect.

and 33). Although ultimately all the nuclei of the sac are alike in appearance as they were before septation commenced, at about the stage of figs. 24 and 32 the micropylar nuclei have often attained the form of those in fig. 24, while those of the lower part of the sac still retain the characters shown in fig. 32. The study of an isolated preparation of a stage very near to this one formerly caused too great an emphasis to be laid upon the differences between the micropylar and the lower nuclei*—differences which are not seen in earlier stages and which quickly disappear.

The vacuoles are large and the bulk of the cytoplasm in which the nuclei are congregated usually comes to lie in contact with one wall of the compartment (fig. 25). As soon as this condition is reached the wall begins to grow upwards in the form of a tube (fig. 25); its extension is closely followed by the cytoplasm enclosing the nuclei. This is the stage represented in fig. 33A of my former paper.† These tubes grow through the macrospore wall into the axial core of the nucellar cone; the lower part of each‡ is highly vacuolated and the bulk of the cytoplasm with the nuclei advances with the tip of the tube (figs. 25 and 26). The lower end soon loses its cytoplasm and appears as an empty cell-wall.§

These tubular upgrowths of the compartment-walls within which the female nuclei are conducted into the nucellar cone were formerly called "prothallial tubes."‖ The study of stages not represented in the earlier collection has shown, however, that the sterile tissue of the sac is not a prothallus and this name must therefore be abandoned. Morphologically they are not strictly comparable with the pollen-tubes, since their walls are not extensions of the endospore. But physiologically the resemblance is very close and it is convenient to denote them by parallel terms. "Embryo-sac-tubes," the name used in this paper, seems to satisfy all requirements. It further possesses the recommendation of being a fairly close approximation to "secondary embryo-sacs," the term adopted by the discoverer of these remarkable structures.¶

The path of the embryo-sac-tube through the nucellus is no doubt mainly determined by the conditions of nutrition. It was formerly suggested** that, owing to the collapse of cells in the axial core of the young nucellar cone, there occurred a loosening of the tissue through which lies the course of the later-formed tubes. This is not confirmed, for in well-preserved material the cells lying above the sac, and not in contact with it, show no signs of degeneration until the upward growth of the tubes commences. While these usually grow upwards and slightly outwards, it

* PEARSON, 1906, pp. 291, 296.
† PEARSON, 1906.
‡ "Bulbous base," *v.* HOOKER, 1863, p. 35.
§ See PEARSON, 1906, fig. 35A.
‖ PEARSON, 1906, p. 289.
¶ HOOKER, 1863, p. 33.
** PEARSON, 1906, p. 288.

sometimes happens that a downward direction is followed (fig. 27), and even more frequently they turn sharply outwards and pass more or less horizontally towards the integument (fig. 10), and may even enter the space between the latter and the side of the nucellar cone. Each tube grows independently of the rest. Of the majority which take an upward direction each continues its growth until a pollen-tube is encountered and the fertilisation of one of the female nuclei is effected. Unfertilised tubes continue to grow until they reach the surface of the nucellar cone. As they advance beyond the normal region of fertilisation they broaden and their cytoplasm becomes much vacuolated. As KARSTEN* has stated, they may even emerge from the nucellar tissue into the micropyle.

In the early condition of the tubes the nuclei lie close together (figs. 26, 28, 29).† Later they usually become separated and are arranged in single file (fig. 31). When the embryo-sac-tube meets the pollen-tube the leading female nucleus is commonly considerably in advance of those behind it (*e.g.*, fig. 53). During the close aggregation of the nuclei in the earlier stages irregularities of form are usually impressed upon them and these are frequently retained up to the time of fertilisation (*cf.* fig. 31). In view of the fusion of the nuclei which normally occurs in the multinucleate compartments of the lower part of the sac, the fact that an entirely similar fusion of all nuclei of an embryo-sac-tube may exceptionally occur is of very great interest. Two such cases are shown in figs. 30, A and B. In the former about six nuclei seem to have participated in the fusion; in the latter the number was probably smaller.

The nuclei of fig. 31 are ready for fertilisation. It is to be emphasised that no nuclear divisions have intervened between the stages of figs. 22 and 31; in other words, the functional female nuclei, although differing in size and to some extent also in staining capacity, are identical with those which occupy the micropylar end of the sac of fig. 21. In most cases there is nothing to indicate that the cytoplasm immediately associated with the nuclei of the embryo-sac-tube has undergone any special degree of organisation; rarely, however, a thin envelope stains more deeply than the general cytoplasm of the tube (fig. 55, Plate 27). There is, however, no sharp line separating the one from the other, and it seems that, as in Gnetum, the female gametes are free nuclei.

Fertilisation may take place in any part of the nucellar cone and in rare cases it may perhaps occur in the axis below the insertion of the integument. But the normal region of fertilisation is situated not far below the line B of the text-figure. Since the embryo-sac-tubes have undergone a considerable growth before pollination (*e.g.*, fig. 27), the sexual fusion cannot normally occur within the limits of the embryo-sac itself, and no indication that this ever happens has been met with.

While in its general behaviour the embryo-sac-tube closely resembles the pollen-

* KARSTEN, 1893.

† See also PEARSON, 1906, figs. 33A, 34, 40.

tube, in one respect there appears to be a remarkable physiological difference between them. The pollen-tube contains a vegetative nucleus—the "tube-nucleus"—usually situated not far from its tip. This normally shows signs of commencing disintegration shortly before the elongation of the tube ceases. While the growth of the tube may continue for some time after its disappearance (figs. 47 and 48), it is nevertheless probable that it performs a definite directive or nutritive function. In the embryo-sac-tube, on the other hand, all the nuclei are apparently equivalent and that which lies nearest the tip normally becomes fertilised (figs. 31, 53, 55, 58), and there are no signs that it is more liable to become degenerate than are those which follow it. It therefore appears that the growth of the tube is independent of a vegetative nucleus.

F. *The Lower End of the Sac and the Endosperm* (Plates 25 and 26, figs. 32–39).

The marking out of the lower three-fourths of the sac into compartments—for the most part multinucleate—and the laying down of cell-walls in the planes of septation take place as in the upper end. It is possible that these processes occur a little earlier in the micropylar end (*cf.* figs. 24 and 32). The lower compartments are less vacuolated and, on the whole, smaller than those which contain the fertile nuclei. The irregularity of form characteristic of them when first established becomes less marked in later stages (*cf.* figs. 23 and 33). The nuclei, at first with the nucleoli more or less obscured by chromatin granules (fig. 23), slowly increase in size and the nucleolus becomes once more visible (fig. 32). As the staining capacity of the wall increases (fig. 33), the nuclei attain their full size and become identical in appearance and reactions with the functionally sexual nuclei of the same sac (fig. 25). One free nucleus of fig. 33 and all those of one compartment of fig. 34 are in mitosis; in the latter case the eight nuclei, five of which are shown, are in the same stage of division. These are the only instances observed of the division of the free nuclei of the sac after the inception of septation, and it is clear from a comparison of the numbers in the sacs of figs. 21, 33, 34, that it is not of general occurrence. A similar simultaneous division of free nuclei occurs in the septate endosperm of *Corydalis cava*;* there, however, it appears not to be limited to a few compartments but to extend through all. Whatever may be the significance of these divisions, they are comparatively rare in Welwitschia.

The nuclei in each compartment now approach one another and accumulate near the centre; in this condition, except for their number, they closely resemble the sexual nuclei of fig. 25. Here, however, they do not remain distinct: at the places of contact the membrane disappears and a gradual fusion occurs (fig. 35); each compartment in this way becomes a uninucleate cell (figs. 35 and 36). The fusion-nucleus usually retains several nucleoli until its first division occurs; in other

* STRASBURGER, 1880, Taf. 2 and 3, figs. 56–60.

cases a single large nucleolus, apparently resulting from the fusion of several smaller ones, is present. The free nuclei in the incompletely septate sac of *Corydalis cava** fuse in a precisely similar manner to form the nuclei of the endosperm cells.

The fusion of the nuclei of the multinucleate compartments of the sterile region of the sac must be effected with considerable rapidity. As a rule, in a cone bearing 40 or more ovules, the earlier stages shown in figs. 35, A, D, are to be found only in two or three. The absence of similar stages from the material collected in 1904 is no doubt to be accounted for by the long period which elapsed between its collection and fixation.† A few cases of the last stages of fusion were figured ;‡ these all occurred near the normally fertile region of the sac and, being associated with the close approximation of the female nuclei in the fertile compartments and the early condition of the embryo-sac-tubes, were misinterpreted as stages in amitotic division from which, it was erroneously suggested, the sexual nuclei resulted.§

Frequently the fusion-nucleus undergoes an immediate mitosis (figs. 33F, 35C, 36). Whether it occurs at once or is delayed for a time, the number of chromosomes is very large and not the same in different nuclei—a fact which is no doubt due to the unequal numbers of constituents taking part in the fusion. In this division the new cell-wall is laid down in a cell plate in the usual manner (fig. 36). As a result of these cell divisions, the lower part of the sac enlarges, passing through the stages outlined in figs. 89, F–H. This primary growth, which occurs before fertilisation, ceases at about the stage of 89H. A more considerable secondary growth commences after fertilisation. The endosperm-tissue, both primary and secondary, is characterised by a marked irregularity in the size of its cells. This seems to be due to the fact that when division occurs the daughter-cells are smaller than those from which they originated, and with successive divisions the difference becomes more marked. Consequently throughout its life the endosperm consists of cells, large and small and of intermediate sizes, irregularly intermingled (fig. 88).‖ In cases in which fertilisation does not take place, the sterile tissue which fills the lower part of the sac (fig. 89H) undergoes little further growth, and eventually becomes disorganised.

An embryo-sac-tube has been figured (fig. 30), in which all the nuclei have fused. If the product is a fusion-nucleus, homologous with that of a primary endosperm-cell, it should, in the ordinary course, undergo mitosis, and proceed to form a tissue. A fusion-nucleus in this position has only been seen twice, and it is clearly of rare occurrence. The structure shown in fig. 37A is contained within an embryo-sac-tube, and is situated a little below the level of the line B in the text-figure. It can only be an extremely abnormal embryo or a small endosperm tissue formed from such a

* STRASBURGER, 1880, Taf. 3, figs. 61–64.

† PEARSON, 1906, p. 266.

‡ PEARSON, *loc. cit.*, figs. 37A, 38A, ♀.

§ PEARSON, *loc. cit.*, p. 292.

‖ See PEARSON, 1906, p. 293, fig. 33B.

nucleus as those shown in fig. 30. Among the hundreds of pro-embryos in various stages that have been examined, the structure and order of development is practically constant. And since, further, no pollen-tube communicates with the cavity in the nucellar cone in which this tube lies, it may safely be concluded that the cell-mass is not derived from an oospore. On the other hand, the seven cells of which it is composed are exceedingly like the smaller cells of the normal endosperm of the same sac (*cf.* figs. 37A and B). The resemblance is seen not only in the size of the cells and in the highly vacuolated condition of their cytoplasm but also in the size of the nuclei, most of which contain more than a single nucleolus. In the absence of intermediate stages it is impossible to be quite certain that this is an endosperm, but there can be very little doubt that this is its true nature. Similar structures similarly situated have been seen in two other cases.

The nuclear fusion which precedes the formation of the endosperm occurs in all those compartments which contain many nuclei. These normally constitute nearly the whole of the lower three-fourths of the sac. But among them are very frequently a few in which the nuclei are in smaller number—six or less. In these, the nuclei sometimes, probably always, do not fuse. These may occur anywhere in the sterile part of the sac, and one or more of them is not uncommonly found close to or at the antipodal end (figs. 38, A, B; 89H). Except for their position these compartments differ in no respect from those of the fertile end of the sac, and it is therefore not surprising that they should behave in a similar manner. The compartment enlarges at the expense of the surrounding cells and vacuoles appear in the cytoplasm. Its wall usually grows upwards, outwards, or downwards, as a tube which eventually penetrates the nucellus like the normal embryo-sac-tube (fig. 89H; text-fig.). Sometimes when it is deeply immersed in the endosperm its growth is equal in all directions, and thus no tube, but merely an enlarged cell, is produced (fig. 38B).* When a tube is formed, the free nuclei and cytoplasm move into it in precisely the same way as in the normal embryo-sac-tube—it is, in fact, an embryo-sac-tube remarkable only for its deep-seated origin. In some cones almost every ovule shows one or more of these abnormally situated tubes; in others they occur in very few. The endosperm-cells lying near them are frequently bi-nucleate, probably as a result of amitotic division. It has not been possible to prove that the nuclei of any of these tubes become fertilised, but their history, behaviour, and appearance leave no room for doubt that they are functionally sexual.

One unpollinated ovule has been found in which no nuclear fusion has occurred, but all the compartments behave like those of the micropylar end of a normal sac; nearly all have produced upgrowing tubes into which their cytoplasm and free nuclei have moved; a few have not formed tubes, but are clearly quite similar to the rest, and are either less advanced in development or have been depleted by their more active neighbours. Most of the tubes have already penetrated the nucellar cone.

* These cells frequently become many times larger than the one figured.

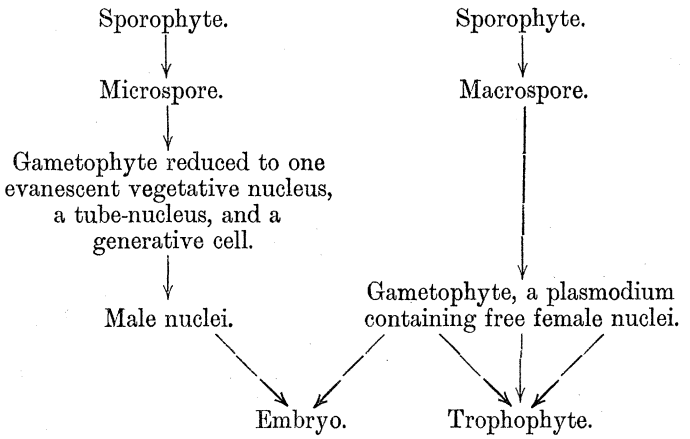
The abnormality of this sac extends also to its form. Its breadth is approximately equal to its length; in bulk it is about as large as a normal sac in a corresponding stage. No more than six nuclei are present in any of the tubes or in the compartments which have not yet formed tubes. In this case there is nothing to indicate the causes which have induced so unusual a course of development. In another cone whose ovules have yielded an exceptionally large number of deep-seated sexual cells, the terminal part of the axis, with its bracts and young flowers, is arrested, presumably as the result of injury—perhaps caused by insect puncture. But however these remarkable abnormalities may have been incited, it is of the greatest interest that under certain circumstances *all* the nuclei in the sac may behave like the functional gametes of a normal sac, at least up to the stage of fertilisation. And since the tubes and their nuclei are all precisely alike—excepting those few whose contents have disappeared—both in appearance and reactions, and are further quite similar in these respects to the tubes and nuclei which in normal ovules become fertilised, it cannot be doubted that these also are potentially sexual.

Another abnormal sac presents a case almost the converse of the last, and the two considered together with the occasional production of a fusion-nucleus within the embryo-sac-tube and the frequent occurrence of compartments containing sexual nuclei among the endosperm-cells leave little doubt as to the potential equality of the sac-nuclei. In this instance the ovule contains two sacs, one above the other, the contiguous surfaces being closely pressed together. In the upper one septation has just commenced (fig. 39) and all the nuclei are free; the lower is considerably more advanced and the endosperm is constituted each of its cells containing a single spherical fusion-nucleus. A normal sac with endosperm-nuclei in this condition would show embryo-sac-tubes as advanced as those of fig. 27. It is extremely interesting to find that where the top of the sac in this case is in contact with the lower wall of the one above it, not only are there no embryo-sac-tubes, but all the nuclei have fused, and the compartments which under ordinary circumstances would have contained free sexual nuclei have become uninucleate endosperm-cells. The significance of this receives emphasis from the fact that a few peripheral compartments of the micropylar region of this sac (which being more advanced is broader than the upper one) still retain their free nuclei and some of them are in an early stage of tube-formation (fig. 39, *e.s.t.*). In other words, where the micropylar end of the lower sac is subjected to a vertical pressure, the compartments have become endosperm-cells by the fusion of their nuclei; where, as at the periphery of this region, there is little resistance to upward growth, this has occurred, and the nuclei are still free and have all the characters of the functionally sexual nuclei of a normal sac. This is regarded as confirming the opinion already expressed (p. 348), that the less crowded condition of the micropylar nuclei which normally precedes septation (fig. 22) is due to the more active growth in length of this end of the sac. When this is

prevented, as in this case by a superincumbent sac, the nuclei do not separate and the compartments consequently contain several nuclei instead of a few, and fusion occurs.

The various abnormalities which have now been described are only explicable on the assumption that the free nuclei in the sac at the time of septation are all alike in every respect—all potential gametes. And since both the history of their origin and their visible characters are also in favour of this conclusion, it may be regarded as proved. The lower part of the sac owes its sterility solely to the fusion of its nuclei, and this is confined to those of its compartments in which fusion has occurred.

An endosperm* formed in this way is clearly not a prothallus, for it is formed as a result of the fusion of sexual nuclei; it is consequently not a part of the gametophyte generation; and further, it does not produce sexual organs or sexual cells. Neither is it a part of the sporophyte generation, for its nuclei contain more than the premeiotic number of chromosomes and it plays no direct part in the production of sporogenous tissue. It possesses a very considerable power of primary and secondary growth; the latter is not manifested uniformly throughout its bulk, but, as will be shown later, is to some extent localised. The endosperm of Welwitschia is therefore an organism showing a degree of specialisation. It is not represented in the lower Gymnosperms or in the Pteridophytes. It is not, however, a newly intercalated generation, for it does not stand in the direct line of the life-cycle of the plant. It is rather a bye-product resulting from the fusion of potentially sexual nuclei, and functioning in the same manner as the prothallus of the lower seed-plants which it replaces in the nutrition of the embryo. There is reason to believe that an endosperm of this character is of general occurrence in the higher seed-plants, and it is necessary to distinguish it from the prothallus. It is therefore proposed to designate it by the term Trophophyte. Its relation to the other stages in the life-history may be thus represented:—



* Endosperm is a physiological term (RICHARD, 1825, p. 353; LINDLEY, 1848, vol. 2, p. 55). It is equally applicable to the prothallus of the gymnosperm as to any other form of nutritive tissue organised within the embryo-sac of a seed-bearing plant.

If under any circumstance two female nuclei only should fuse, the fusion-nucleus would possess the number of chromosomes characteristic of the sporophyte and a normal embryo might perhaps result—as when two post-meiotic nuclei of vegetative cells fuse in the prothallus of *Lastrea pseudo-mas* var. *polydactyla*, WILLS.* Evidence that this occurs in Welwitschia has been searched for in vain. In view of the similar conditions of the Gnetum sac—to be discussed later—it is possible that the supposed parthenogenetic embryo in *G. ula*† may originate in the fusion of two of the sac-nuclei.

A comparison of the endosperm of Welwitschia as regards its manner of origin with that of Ephedra on the one hand and of Gnetum on the other leads to important conclusions. There can be no doubt that in Ephedra the endosperm is a true prothallus formed in the same way as that of the Conifers, the Cycads, and the higher Pteridophytes. Its development has been studied in four species‡ and, in all, the free-nuclear embryo-sac becomes septate as a result of the formation of centripetal tubular alveoli. In the later stages of its development multinucleate cells are met with,§ as in some other Gymnosperms.‖ In Taxus, many of the endosperm-cells, all of which are initially uninucleate, become multinucleate—whether by direct or indirect division is not certain. These cells may eventually contain as many as 16 nuclei. After this nuclear fusion occurs, and the number of nuclei in each cell is, in consequence, reduced.¶ This is succeeded by their degeneration. JAEGER observes that the degeneration occurs first in those endosperm cells which border upon the archegonia or embryo. Whatever may be the significance of the multiplication followed by fusion and degeneration of the nuclei of endosperm-cells, it is clearly a process in no way connected with the nuclear fusion which produces the Welwitschia endosperm, and its occurrence does not lessen the fundamental difference which exists between the prothallus of Ephedra and the trophophyte of Welwitschia. At the micropylar end of the prothallus of Ephedra, as of the lower Gymnosperms, there arise sexual organs whose homology with the archegonia of the Pteridophytes cannot be questioned. Welwitschia and Ephedra are therefore very widely separated by the character of the endosperm and of the sexual apparatus.

It is unfortunate that the development and the minute structure of the endosperm of Gnetum has been but little investigated. The species that have been studied fall into groups. In the one which appears to be most numerously represented,** endo-

* FARMER and DIGBY, 1907.

† LOTSY, 1903.

‡ STRASBURGER, 1872, Taf. 16, fig. 52; SOKOLOWA, 1891, fig. 22 and *sqq.*; JACCARD, 1894, p. 17; BERRIDGE and SANDAY, 1907, p. 129.

§ JACCARD, *loc. cit.*, fig. 48; STRASBURGER, 1880, p. 106.

‖ JAEGER, 1899, pp. 258 and *sqq.*, Taf. 17, fig. 29; STRASBURGER, 1880, p. 107; LAWSON, 1904, p. 426.

¶ JAEGER, *loc. cit.*, and fig. 30.

** KARSTEN, 1892, 1893.

sperm is not formed before fertilisation. The embryo-sac becomes filled with free nuclei embedded in cytoplasm, as far as can be judged, in the same way as in Welwitschia, except that in the early stages there is normally a large central vacuole. All the nuclei after the last divisions have occurred are said to be alike in origin and potentially sexual in function. Some of these are fertilised. As soon as this has occurred the lower part of the sac becomes septate. According to KARSTEN'S figure of *G. edule** (*G. Rumphianum*),† the compartments are very irregular in form, and most of them are multinucleate. In *G. ovalifolium* "in vielen Endospermzellen sieht man zwei Kerne; es kommen auch häufig weit mehr als zwei auf eine Endospermzelle bei der *ersten Zerlegung* des Plasmabelages."‡ It is implied that these compartments later become uninucleate cells and afterwards undergo division. Nuclear fusion is not recorded, but if it does not occur these species of Gnetum must show still a third method of endosperm-formation differing alike from those which are characteristic of Ephedra and Welwitschia respectively. This is exceedingly improbable.

Gnetum Gnemon must be separated from the species studied by KARSTEN, on the ground that an endosperm is formed, at least in many cases, before fertilisation. There is every indication that at the end of the free-nuclear condition of the sac the nuclei are equivalent in every respect. Those of the lower part of the sac form a sterile tissue, which later fills the sac and nourishes the pro-embryos, while any of those in the upper part may become fertilised. LOTSY gives little detail as to the manner in which this sterile tissue is formed, but the following sentence is significant:—"We notice that in fig. 29, Plate 4, several cells contain as yet a number of nuclei, while in fig. 34, Plate 5, this has already been remedied, every cell containing but one."§ The nature of the remedy has not been ascertained, but in view of what occurs in Welwitschia it is reasonable to conclude that here also it is nuclear fusion. After fertilisation "a greater or smaller number of the nuclei of the fertile part of the embryo-sac surround themselves with a denser protoplasm, a membrane, and thus form cells."‖ These "retarded prothallium cells" do not persist, being eventually obliterated and replaced by the endosperm growing up from below. The more active growth of the latter tissue is no doubt the result of the stimulus due to the fusion of its nuclei.

One other feature of the endosperm of *G. Gnemon* described by LOTSY possibly has a bearing on this discussion. There occur occasionally at the summit of the endosperm groups of cells of a remarkable form, "which one is compelled to consider as rudimentary archegonia."¶ One of these is shown in section in fig. 62.¶ One of

* KARSTEN, 1892, fig. 9A.
† KARSTEN, 1893, p. 338.
‡ KARSTEN, *loc. cit.*, p. 372.
§ LOTSY, 1899, p. 98.
‖ *Loc. cit.*, p. 97.
¶ *Loc. cit.*, p. 98.

the cells of the group is much larger than the rest, and contains several nuclei. Its general similarity to one of the compartments of the endosperm of Welwitschia in which nuclear fusion has not occurred is certainly striking. It must be admitted, however, that this interpretation is not confirmed by other figures of similar structures (*cf.* figs. 55 and 56),* though it is not clear that even in these cases the cells are originally uninucleate.

It is a reasonable inference that in the species in which it is formed after fertilisation, as well as in *Gnetum Gnemon*, in which its organisation is usually delayed, the endosperm of Gnetum is a trophophyte essentially similar to that of Welwitschia.

G. *Germination of the Pollen-grain and Growth of the Pollen-tube* (Plates 26 and 27, figs. 40–52).

The exine splits into two lobes,† and may be thrown off entirely as in Ephedra.‡ The tube-nucleus passes into the growing intine in advance of the generative cell. The latter increases considerably in size during the early stages of germination (figs. 40–43, *g.c.*),§ and lies quite free in the cytoplasm of the pollen-grain. As it leaves the body of the grain it becomes elongated in the direction of the axis of the tube and narrower in proportion. The shape of its nucleus is usually affected in the same way (fig. 41). The anterior end of the cell is bluntly pointed, while posteriorly it is thrown out into a number of irregular lobes. Both these characteristics are usually found up to the time of fertilisation. The nucleus possesses a single large nucleolus.

The pollen-tube usually enters the nucellus at the summit; sometimes it continues to grow in the micropylar fluid between the integument and the side of the nucellus; it occasionally passes beyond the line B in the text-figure before turning sharply inwards and penetrating the nucellus. Soon after entering the nucellus both the generative cell and its nucleus undergo a further increase in size (fig. 43). In front of the cell there is usually a vacuolar space. The tube-nucleus frequently lies, in these early stages, about midway between the tip of the tube and the front end of the generative cell (figs. 43A and 46); it is often, however, almost in contact with the latter (fig. 43B). Its form is somewhat irregular, but it is most frequently more or less elongated and crescent-shaped (figs. 43, 45, 46). In the young tube it is usually sharply differentiated from the surrounding cytoplasm, but as it approaches the level of the line B (text-figure) its outline becomes indistinct, and below this line the signs of its disintegration are always well marked (fig. 50). Its nucleolus, which is frequently as large as that of the male nucleus, is the most persistent part of it. At the time of fertilisation even this is rarely recognisable.

* Lotsy, 1899, p. 98.
† Pearson, 1906, fig. 17.
‡ Jaccard, 1894.
§ *Cf.* Pearson, 1906, fig. 16.

The identification of the male and female nuclei just prior to the process of fertilisation is often difficult, even when the latter is quiescent in a large oosphere. Here the male nucleus is easily distinguished by its cytoplasmic sheath, its constantly large nucleolus and, very frequently, by its elongated form. More serious difficulty is occasioned by the resemblance between some forms of the tube-nucleus and the female gametes. In view of the fact that fertilisation occurs within the male cytoplasm, it becomes especially important to distinguish the one from the other. In well-fixed material the female nucleus is approximately spherical in form and its nucleolus is almost invariably smaller than that of the tube-nucleus. More important, however, is the fact that before contact between the two tubes is effected the tube-nucleus is either already absorbed or at least shows very distinct signs of deterioration. Occasionally, however, undoubted female nuclei have been seen which very closely resemble a common form of the tube-nucleus (*cf.* figs. 29 and 46). While these are almost certainly deteriorated in fixation their occasional occurrence renders necessary the exercise of considerable caution in identifying as female a nucleus lying just in front of the male cytoplasm after communication between the two tubes has been established.

While the direction of growth of the embryo-sac-tubes is in general more or less away from the axial line of the nucellar cone, that of the pollen-tubes is rather towards it. The meeting of the two sets of tubes occurs usually near the axis of that part of the nucellar cone which lies between the lines B and C (text-figure). Fertilisation may be effected, however, in any part of the nucellus lying above the top of the embryo-sac. Pollen-tubes which do not come into contact with embryo-sac-tubes in the nucellar cone still continue their downward growth. Instances of this are not common, for the lower part of the nucellar cone is so thoroughly perforated by embryo-sac-tubes, always more numerous than the pollen-tubes, that their meeting is almost ensured; added to this there is also the probability that they are definitely attracted towards one another by chemiotaxis (fig. 53). Nevertheless a pollen-tube will rarely find its way down into the evacuated fertile region of the sac almost to the top of the endosperm itself, where its contents are no doubt absorbed by the actively growing pro-embryos. In any case the nuclei of such a tube can exert no influence upon the growth of the endosperm; apart from the very small number of cases in which they are brought into its vicinity, the primary endosperm-nuclei are fully formed and cell-division has always commenced before pollination occurs. In one ovule which contains a number of embryos, a pollen-tube, having grown through the region occupied by the embryo-sac-tubes, has reached a point in the chalazal tissue about half-way between the base of the endosperm and the insertion of the perianth. Its course lies entirely through the nucellar tissue; it is not all shown in one section but is indicated by dotted lines in fig. 47. Near its tip is the bi-nucleate generative cell (figs. 47 and 48, *g.c.*); the tube-nucleus has disappeared.

At the time of pollination the cells of the nucellar cone are packed with spherical starch-grains which stain readily in diamant fuchsin. In this condition they are seen to be composed of three or four simple grains, each of which has in section the form of the sector of a circle and shows a minute central hilum (fig. 42). Their compound structure is not brought out by any of the other stains used and they were formerly described as simple and concentric.* The starch-grains of the endosperm have the same structure.

Soon after entering the nucellar cone the nucleus of the generative cell divides (figs. 44 and 45). The spindle is indistinct; its axis is parallel with that of the cell. The two nuclei resulting from this mitosis are at first spherical in form and lie near together at either end or in the middle of the cell (figs. 46, 49, 50, etc.). They undergo no further division; both are potentially sexual.

The bi-nucleate generative cell shows a considerable range of differences in its later behaviour. The nuclei very frequently exhibit a tendency to elongate (figs. 51, 54, 55, etc.), which may become very pronounced (figs. 59 and 60). Whatever their form, the leading nucleus is usually smaller than the one behind it (figs. 46, 48, 51, etc.). Sometimes both persist until the time of fertilisation (fig. 57); when the cytoplasm of the cell remains intact one of the two nuclei frequently—perhaps usually—breaks down; this may be either the anterior (fig. 52) or the posterior (fig. 59). Sometimes the cytoplasm of the cell becomes separated into anterior and posterior portions, each containing one nucleus (fig. 60A). It is probable that this, when it does occur, is a purely mechanical effect of the strains caused by the passage of the cell through a narrow and commonly tortuous passage. Since these uninucleate masses of cytoplasm are not homologous with the male cells of the lower Gymnosperms, the term "generative cell" is used indifferently for the cytoplasmic sheath, broken or entire, enclosing, in the former case, one nucleus, and in the latter, one or two nuclei. At the time of fertilisation, then, the generative cell may contain either two male nuclei or a single male nucleus, the other having disintegrated, or, on the other hand, it may have broken into two parts, each containing a single potentially sexual nucleus.

The male nucleus is characterised by a single nucleolus which is always distinctly larger than that of the female. In most cases there is a considerable quantity of extra-nucleolar chromatin. Extra-nuclear granules staining deeply in diamant fuchsin are also usually present. In no case does the nuclear body show any signs of a spiral curvature.

The development of the male gametes from the generative cell differs remarkably from the corresponding series of events in Ephedra and the lower Gymnosperms; it appears, however, to be quite similar to those which occur in some species of Gnetum.† In the omission of the division which cuts off the "stalk-" or "wall-cell" and in the fact that the male nuclei are free within the generative cell, Welwitschia and

* PEARSON, 1906, p. 292.

† KARSTEN, 1893, figs. 59–64.

Gnetum show a close approach to the condition of many Angiosperms.* The last gymnospermic feature retained in the structure of the pollen-grain is the reduced and evanescent prothallial cell,† such as is also described for a few Angiosperms.*

H. *Fertilisation* (Plates 27, 28, and 29, figs. 53–67).

While in the early stages of the pollen-tube the tube-nucleus is usually more or less in advance of the generative cell and some distance behind the tip of the tube (fig. 46), when the latter crosses the line B in the text-figure they lie near together and not far from the apex (fig. 50). By this time the tube-nucleus has lost the greater part of its chromatin and its outline is commonly indistinct. As the tube advances, the body of the nucleus becomes less distinct and finally the nucleolus disappears. The generative cell, or the leading portion of it, then comes to lie just within the tip of the pollen-tube (figs. 51 and 55) or a very little way behind it (fig. 63).

As the embryo-sac-tube approaches the pollen-tube its leading nucleus is almost invariably considerably in advance of the rest. It is on this account and owing to the complex of tubes perforating the lower part of the nucellar cone that it is frequently impossible to identify the other nuclei of a tube whose leading one has become fertilised. After its fertilisation it seems that the others of the same tube degenerate (figs. 65 and 67). The fertilisation of more than one of the female gametes of a tube is theoretically possible, but no instance in which it has certainly occurred has been observed. A possible case is that of the tube whose upper end is shown in fig. 56 and which is continuous below with a cavity in the nucellus in which lies a two-celled pro-embryo. If the nucleus "♀?" in this figure is correctly identified as a female gamete,‡ it is probable that the pro-embryo results from the fertilisation of a second female nucleus in the same tube.

Before the nucellar tissue which separates them has become disorganised, a pair of tubes are often observed to be growing very definitely towards one another (fig. 53), from which it may be inferred that they both or one of them is under the influence of a directive force. Since the most active growth of the embryo-sac-tube occurs before pollination it might be expected that the attractive influence emanates from it. On the other hand, several cases have been seen in which the tip of the embryo-sac-tube comes into contact with the pollen-tube, not at its apex but laterally in the neighbourhood of the generative cell (figs. 57 and 63); this suggests that the growth of the embryo-sac-tube is rather directed towards that part of the pollen-tube in which the generative cell lies. The walls at the place of contact break down and their contents are placed in communication. The leading female nucleus passes directly into the generative cell within which fertilisation takes place. This very remarkable behaviour

* Coulter and Chamberlain, 1903, pp. 133 and *sqq.*

† Pearson, 1906.

‡ It is very improbable that this is an unusually persistent tube-nucleus, but this is a case in which its identity is somewhat doubtful.

of the female gamete has been carefully ascertained and there can be no doubt that it is normally antecedent to the nuclear fusion. Fig. 60A shows both the male nuclei, the generative cell having broken in the middle; within the cytoplasm of the leading portion of it, lying immediately in front of its nucleus, is an undoubted female nucleus (fig. 60B). In this case the lower part of the embryo-sac-tube comes immediately from a complex of similar tubes and its other nuclei cannot be certainly identified. The male nucleus of the hinder part of the generative cell shews signs of incipient degeneration. A similar case is shown in figs. 58A, B, C; here, in three of four successive sections, are shown: (1) a male and a female nucleus within the generative cell (A); (2) the same male and a second female nucleus behind the first one and not within the generative cell (B); (3) a third female nucleus considerably lower down in the same embryo-sac-tube (C). The posterior male nucleus has disappeared. Embryo-sac- and pollen-tubes in communication are shown in two successive sections in figs. 62 and 63. In the former a female nucleus is in contact with the male within the generative cell; in the latter there is a second female nucleus at a lower level in the same embryo-sac-tube. Other cases in which the female nucleus is clearly within the generative cell are shown in figs. 61 and 64. In the latter the second male nucleus is not to be found; in the former it is probably represented by a vestige of chromatin in the hinder part of the generative cell.

The cytoplasm of the generative cell is the most conspicuous structure within the pollen-tube; the general cytoplasm of the tube as well as all that of the embryo-sac-tube is, when fertilisation occurs, uniformly distributed and vacuolated. If, then, the fusion of the gamete were ever effected outside the limits of the generative cell, the remains of the latter would certainly be visible for a time in the neighbourhood of the oospore. A great many oospores have been examined, but such an extraneous mass of cytoplasm has never been found in the neighbourhood of any of them except in a few cases in which it contained a male nucleus and was clearly the second half of the generative cell. The cytoplasm of the oospore is so massive (figs. 65–67) and that of the pollen-tube and embryo-sac-tube so lacking in organisation, that the former must be derived very largely, if not entirely, from the generative cell. Since in the young oospore the cytoplasm frequently does not stain uniformly (fig. 65), it is probable that some accompanies the female nucleus from the embryo-sac-tube. But there is no doubt that in Welwitschia the male cytoplasm plays a much more prominent part in the process of fertilisation than has been found to be the case in other Gymnosperms.*

In some cases, at least (*e.g.*, figs. 57, 63, 64), the generative cell certainly does not leave the pollen-tube, but the female nucleus passes out of the embryo-sac-tube to enter it. The latter is identified in each case by the presence within it of a second female nucleus below its junction with the pollen-tube. Owing, however, to the large number of tubes and their very devious courses, it is usually impossible to determine with certainty in which of the two tubes the generative cell at the time of fertilisation,

* COKER, 1907, p. 6.

or the oospore, lies. But no case has been found in which fertilisation clearly occurred within the embryo-sac-tube and it is probable that it always takes place while the generative cell lies within the pollen-tube. This would account for the connection which HOOKER constantly found between the pollen-tube and the primary suspensor of the pro-embryo.*

As the male and female nuclei come into contact (figs. 61 and 63), a concavity frequently appears in the former. Sometimes however it seems to be the male nucleus which impresses the surface of the female. The fusion (fig. 64) is rapidly completed, but the nucleoli remain distinct for a time (figs. 65 and 66). They are still to be distinguished by their difference in size. At first the body of the nucleus is differentiated into two parts, of which one stains more deeply than the other (fig. 65); in later stages this difference has disappeared (figs. 66 and 67). A cell-wall is formed just within the limits of the generative cell (fig. 67). In some cases a second female nucleus, in a condition of degeneration, is included within the delimited cytoplasm (fig. 65).

A resting nucleus is thus formed as a result of fertilisation and in the first mitosis the paternal and maternal chromosomes are not to be distinguished (figs. 68 and 69). This is the case also in Lilium† and other Angiosperms, while in Pinus‡ and perhaps in Gymnosperms generally,§ a resting fusion-nucleus is not formed. LAND'S|| account of *Ephedra trifurca* suggests that Ephedra and Welwitschia are in agreement in this respect, but the first mitosis in the oospore of the former has not been described.

The details of fertilisation are insufficiently ascertained in Gnetum, in which the fusion of the male and female nuclei has not been observed. In *Gnetum Gnemon* the male nuclei are free in the cytoplasm of the pollen-tube, and in this condition enter the embryo-sac.¶ In other species, however, the male gametes are surrounded by the well-defined cytoplasm of a generative cell ;** they are discharged into the embryo-sac, each surrounded by cytoplasm††—presumably that of the generative cell. The nuclei are somewhat elongated and narrowed towards the anterior end, as is so frequent in the case of Welwitschia.‡‡ Whether the cytoplasm accompanying them takes part in the organisation of the oospore was not determined, though KARSTEN formerly inclined to the opinion that this was the case.§§

* HOOKER, 1863, Plate 10, figs. 16–19.
† MOTTIER, 1904, p. 176.
‡ BLACKMAN, 1898.
§ DAVIS, 1905, p. 232.
|| LAND, 1907.
¶ LOTSY, 1899, p. 94.
** KARSTEN, 1893, fig. 65, etc.; LOTSY, *loc. cit.*
†† KARSTEN, *loc. cit.*, p. 379.
‡‡ KARSTEN, *loc. cit.*, fig. 71.
§§ KARSTEN, 1892, p. 224.

I. *The Pro-embryo* (Plates 29 and 30, figs. 68–87).*

The number of oospores present in a nucellus is very variable. It is rarely so few as one, and probably never exceeds the number of the pollen-tubes. It is not uncommon to find as many as a dozen pro-embryos in an ovule.

After the formation of the single nucleolus, the oospore and its nucleus increase in size and become elongated in the direction of the axis of the pollen-tube within which it lies. The boundary of the oospore-cytoplasm approximately coincides with that of the original generative cell (fig. 67). Where, however, both the male nuclei have persisted, only the anterior part of the generative cell contributes cytoplasm to the oospore. This is probably the reason of some differences in size frequently exhibited by oospores in the same nucellus. When the wall is completed the elongation of the cell becomes more pronounced and vacuoles appear in the cytoplasm on the micropylar side of the nucleus (figs. 68 and 69). This growth is certainly for the most part in a downward direction. But the frequent occurrence of an irregular projection at the micropylar end probably indicates that at first the cell elongates in both directions. Since, in later stages, the micropylar end of the pro-embryo is always found in contact with unbroken nucellar cells, it seems that the upward growth continues until a *point d'appui* is reached. A very similar projection at the micropylar end of the elongating oospore of *Gnetum Gnemon* is described and figured by LOTSY.† The cells at first lie entirely within the canal excavated by the pollen-tube and embryo-sac-tube. In many cases the pro-embryo continues its course down the canal formed by the embryo-sac-tube, which formerly brought the female nucleus which has given rise to it. On the other hand, it frequently bores a new path through the hitherto uninjured nucellar tissue. Gravity can have little if any influence upon its direction of growth. Apart from the fact that the female cones, and with them the ovules, are usually inclined at a considerable angle to the vertical, there are circumstances in which the pro-embryo will grow directly upwards towards the micropyle.‡ This has been observed only in a few cases, in all of which fertilisation has occurred above the line B (text-figure). It would seem therefore that the direction of growth is determined mainly by conditions of nutrition and, further, the downward course will usually be that of least resistance. It may be that oospores situated near the micropyle are within the range of influence of the sugar left by the drying up of the micropylar fluid.

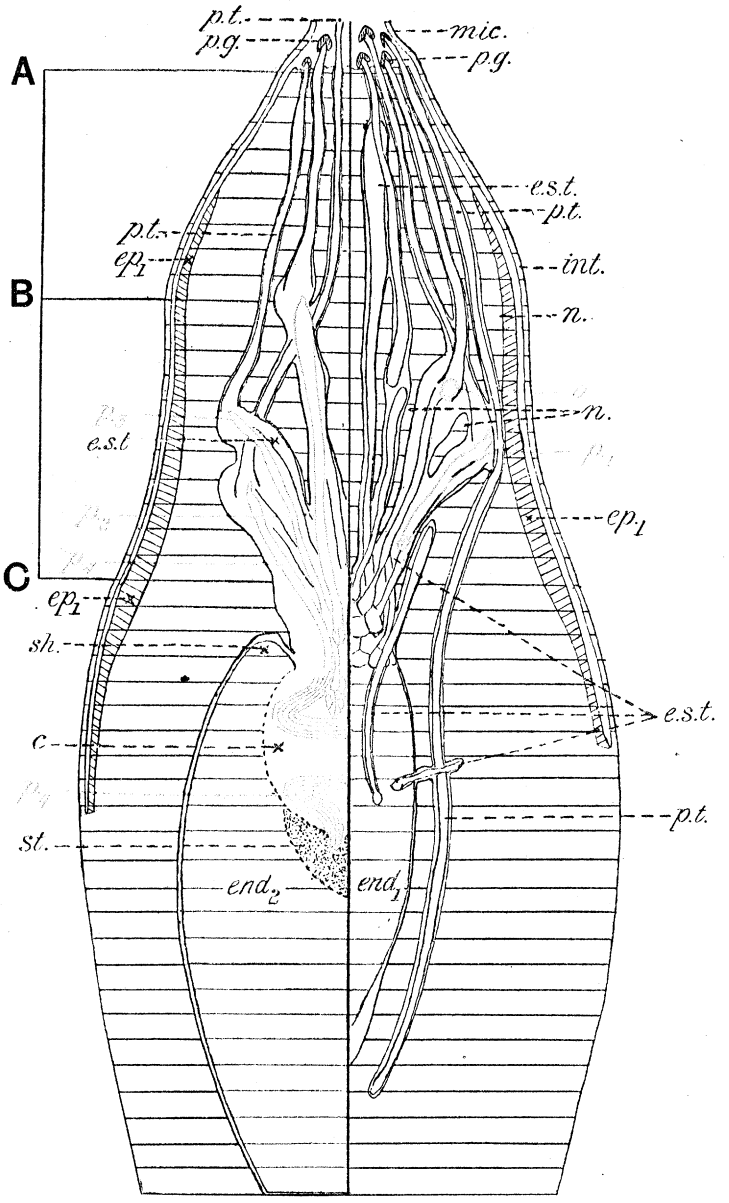
As the cell elongates the nucleus moves downwards and, when not far from the lower end of the tube, undergoes mitosis (figs. 68 and 69). At the conclusion of this division a wall is formed, transverse to the axis, dividing the oospore into two cells, of which the lower one is much smaller than the upper (fig. 71). The origin of this

* Some of the stages described below are figured in outline by STRASBURGER (1879, Taf. 22).

† LOTSY, 1899.

‡ This sometimes also occurs in Gnetum (LOTSY, 1899).

transverse wall has not been certainly ascertained. During the anaphase of the mitosis (fig. 69) there is no visible cell-plate. Fig. 70 shows a case in which the peripheral part of the transverse wall is very distinct while in the centre, between



Text-figure.—A diagrammatic longitudinal section, showing halves of two ovules, one at the stage of fertilisation (on the right), the other containing advanced pro-embryos (on the left). The breadth is more highly magnified than the length.

c. = cavity in endosperm caused by the entrance of several pro-embryos; *end.*$_1$, *end.*$_2$ = primary and secondary endosperm; *ep.*$_1$ = outer (epithelial) layer of nucellar cone (*cf.* figs. 9, 10, *ep.*$_1$); *e.s.t.* = embryo-sac-tube; *int.* = integument; *mic.* = micropyle; *n.* = nucellus; *o.* = oospore; *p.*$_1$ = 3-celled pro-embryo (*cf.* fig. 73); *p.*$_2$ = 9-celled pro-embryo (*cf.* fig. 76); *p.*$_3$ = later pro-embryo (*cf.* fig. 81); *p.*$_4$ = advanced pro-embryo (*cf.* fig. 85); *p.g.* = pollen-grain; *p.t.* = pollen-tube; *sh.* = shoulder of secondary endosperm; *st.* = region of the secondary endosperm in which the cells contain more starch than is present elsewhere.

the nuclei, it cannot be traced. It is therefore probable that, as in *Ephedra trifurca*,* it arises as an ingrowing ring from the wall of the oospore.

The upper of the two cells of which the pro-embryo now consists undergoes no division; it attains a considerable length as the "primary" suspensor and later as the axial part of the "secondary" suspensor. The lower cell quickly divides, to give rise to a group of four initial cells from which are later formed: (*a*) twenty-four "cortical" cells of the secondary suspensor; (*b*) the embryonic plate of eight cells; (*c*) a ring of sixteen cells around the latter; (*d*) a terminal "cap" of eight cells.

As the primary suspensor elongates, pushing the lower cell down in front of it, its cytoplasm becomes more vacuolated and its nucleus maintains a position not far from its lower end (figs. 71 and 72). The terminal cell now divides longitudinally (fig. 72) and the two resulting cells (fig. 73) divide again in a plane at right angles to that of the first division. At the lower end of the primary suspensor there is now a group of four beak-like initial cells (fig. 74, *i.c.*). Each of these divides in a plane at right angles to the two former divisions. One cell undergoing this division is shown in fig. 75; the other three, in practically the same phase, lie in the next section, but are somewhat displaced in preparation. There results a group of eight cells in two tiers; those of the upper tier are smaller than those of the lower (fig. 76). The former divide by radial walls parallel to the axis of the pro-embryo; this division is in progress in fig. 77. The upper tier now has its full complement of eight cells; it later gives rise to the inner cortical ring of the secondary suspensor; it is therefore conveniently referred to as the "inner cortical tier" (*i.c.t.*, fig. 78). By this time the elongation of the primary suspensor has brought the terminal group of cells into the region formerly occupied by the fertile end of the embryo-sac (text-fig. $p_1\,p_2$).

The four terminal initial cells divide by radial longitudinal walls meeting in the axis of the pro-embryo. Henceforward the initial group contains eight equal cells (fig. 86A). Each of these cuts off a cell by an oblique wall (fig. 78, A and B). The cells so separated form a ring lying above and outside the initial cells and beneath the inner cortical tier (fig. 79). The eight cells of this ring further divide by radial longitudinal walls producing the sixteen initial cells of the outer cortical ring (figs. 80, 86C, *o.c.r.*).

The next stage is marked by the further division of the eight terminal initial cells by walls transverse to the axis of the pro-embryo (fig. 80). There is thus formed a plate of eight cells (figs. 80–82, 84, 85, 86B), which lie within the outer cortical ring and form what is provisionally called the "embryonic plate" (E).

When this stage is reached the terminal cell-group of the pro-embryo has usually penetrated the upper part of the endosperm and the formation of the secondary suspensor commences (text-fig., p_3). The first sign of this is the elongation of the

* LAND, 1907, figs. 14 and 15.

eight cells forming the inner cortical tier (*i.c.t.*), which has already begun in fig. 80. The direction of growth of these cells is that of the elongation of the whole pro-embryo. The initial stage of their elongation is marked by the appearance of a vacuole in the proximal part of the cytoplasm. The growing region is clearly intercalary and is situated at the angle where the cell curves in under the primary suspensor. In view of HOOKER'S figures,* it is to be emphasised that these eight cells together with the distal end of the primary suspensor grow on together as a tissue-unit; they are not isolated "embryonal tubes" with independent growth in a backward direction as has been believed. The same is true of the cells of the outer cortical ring.

While the eight cells of the inner cortical ring are in an early stage of elongation (figs. 80 and 81, *i.c.r.*), the nucleus of the primary suspensor-cell shows signs of disintegration and in later stages cannot be distinguished (fig. 82). Meanwhile the eight terminal initial cells undergo a division similar to that which formerly cut off the outer cortical ring. A ring of eight cells, afterwards by radial longitudinal division increased to 16, is thus formed lying above and outside the initial cells and beneath the outer cortical ring. These 16 cells are unchanged in the most advanced embryo yet found. One of HOOKER'S figures (Plate 10, fig. 25) suggests that they may later give rise to a third ring of cortical suspensor cells. They will be referred to here as the "lower ring" (figs. 81–85, 86B, "*x*").

The 16 cells of the outer cortical ring now begin to elongate in precisely the same manner as did those of the inner ring (figs. 82, 83, 84, *o.c.r.*). Fig. 84, an oblique section, shows four cells of the inner cortical ring, two of the outer ring, one which may belong to either, five embryonic cells, two cells of the "lower" ring, and four of the terminal cells. The outer cortical cells are considerably more elongated in fig. 85, but no further change has occurred. This figure represents the most advanced pro-embryo seen. It recalls that of *Araucaria brasiliana*.† Until it is possible to follow its development further it will be best to assume that the terminal group of eight cells formed when the lower ring-cells were cut off from the initial cells (figs. 81, etc., "*c*") is homologous with the "embryo-cap" of Araucaria; like the latter, it is certainly absorptive in function (see fig. 85). It further affords mechanical protection to the plate lying above it, which, from analogy with Araucaria, is regarded as the initial embryonic group (E).‡

The relations of the cells composing the pro-embryo of the stage of fig. 85 will be made clear by reference to the series of diagrams of fig. 86 and to fig. 87 representing transverse sections at successively higher elevations. Fig. 86A is taken from

* HOOKER, 1863, Plate 10. (These figures were obtained from badly preserved material—*loc. cit.*, p. 36.)

† STRASBURGER, 1879, Taf. 20, figs. 66–71.

‡ More advanced stages are figured by STRASBURGER (1879, figs. 87–93); but the later development of this plate is obscure (E).

a section at the level of the line marked "A" in fig. 85. It shows the eight cap-cells. A section through the level of "B" (fig. 86B) shows, internally, the eight cells of the embryonic plate (E) which are surrounded by the 16 cells of the lower ring ("*x*"). The section of fig. 86C passes through the cells of both outer (*o.c.r.*) and inner (*i.c.r.*) cortical tiers below the end of the primary suspensor (fig. 85, at the level "C"). In fig. 87 (see fig. 85D) is shown a transverse section through the secondary suspensor which, in the most advanced stage seen, always consists of 25 cells, viz., the primary suspensor surrounded by eight inner cortical cells outside which are 16 outer cortical cells. The secondary suspensor is circular in section, while the terminal cell-group is more or less elliptic. The form of the latter does not appear to be influenced by the flattening of the endosperm, which is usually well marked by the time that it is penetrated by the pro-embryos.

When only one pro-embryo is present it seems usually to pursue a fairly direct course towards the centre of the endosperm. When, however, as more commonly occurs, several pro-embryos are formed, the core of the upper third of the endosperm becomes totally broken down (text-fig. C). In the cavity thus produced by the activity of their own absorptive powers the growing ends of the pro-embryos pursue a somewhat spiral course and a section frequently shows the broken-down tissue of the endosperm almost replaced by a most confused arrangement of suspensors cut in all possible planes.* As a rule one outpaces the rest and thrusts its terminal group of cells into the heart of the endosperm (text-fig., p_4). The mature seed apparently never contains more than one embryo, and it may be presumed that the pro-embryonal group which first reaches the solid endosperm is destined to survive. It is now immersed in a tissue whose cells contain a greater accumulation of starch than any other part of the endosperm. This region is not so markedly differentiated until one or more embryos have entered the endosperm.

The convolutions of the suspensor are so numerous that only short lengths of it are obtained in any one section. It is therefore very difficult to determine whether transverse walls are formed in the cells composing it. Both in the primary suspensor and in the cortical cells the nucleus is always found not far from the pro-embryonal group (figs. 82 and 85); it has never been seen in division and more than one has not been found in the same cell. What at first sight appear to be oblique walls occur only when the suspensor has suddenly changed its direction. It may therefore be inferred that normally the cells of the suspensor do not divide by transverse walls, but the possibility that these may occasionally occur, as in Gnetum,† is not precluded. The upper (older) parts of these cells are without cytoplasm; their walls react strongly to cellulose stains and show no trace of cutinisation.

* The cavity "filled with a coiled mass of tubular structures" found at the apex of the endosperm of Gnetum (BOWER, 1882, p. 278) is no doubt of a similar nature.

† BOWER, 1882, p. 279; LOTSY, 1899, Plate 10, fig. 57.

The later stages of the Welwitschia embryo and the details of the whole development of the pro-embryo of Gnetum must be investigated before any close comparison between the embryonic stages of the three genera can be instituted. But in so far as the facts are known, they indicate a closer degree of affinity between Welwitschia and Gnetum than between either of these genera and Ephedra.

In Ephedra, the oospore-nucleus undergoes three series of divisions, and each of the eight resulting nuclei may organise a cell which gives rise to a pro-embryo. In some species of Gnetum, the oospore is said to behave in a somewhat similar manner.* In *G. Gnemon* and some other species and in Welwitschia the oospore produces a single pro-embryo. In *Ephedra altissima*† and in *E. trifurca*‡ the formation of the primary suspensor corresponds fairly closely with that of Welwitschia. What LAND calls the "secondary" suspensor of *E. trifurca* is, however, very different from that described above and is apparently of the same nature as the Cycadean suspensor. In Ephedra, the intraseminal development of the embryo is completed while the ovule is still attached to the parent plant. Indeed, under favourable conditions, germination may occur before the strobilus falls.‡ In Gnetum, on the other hand, the development of the embryo itself does not commence until after the seed has fallen.§ None of the cones of Welwitschia that have been examined have attained their full size, but the most fully developed pro-embryos seen (fig. 85) are not confined to the largest ovules studied. It is therefore quite possible that here, as in Gnetum, the embryo develops in the fallen seed before germination. The only seeds that have been available for examination which contained large and fully developed embryos were lying on the ground and had probably been in that condition for six months. All are agreed that, of the three living genera, Ephedra is the most nearly allied to the lower Gymnosperms. If the group is monophyletic, it is not a little remarkable that its highest members should, in the deferred development of the embryo, have reverted to conditions so characteristic of primitive seed-bearing plants.

Beyond the fact that in Gnetum the oospore elongates to form a tubular suspensor, we have little information as to the early changes following fertilisation.‖ It is probable that, as in Ephedra and Welwitschia, a primary suspensor with an initial cell at its tip is formed, as a result of the first division of the oospore-nucleus, though BOWER states that no terminal cell is cut off before germination.¶ This tubular suspensor is described as occasionally undergoing branching, a condition which has not been met with in Welwitschia. After the appearance of a few anticlinal walls in the terminal cell of the pro-embryo, "the peripheral cells of the group thus formed grow laterally along the surface of the suspensor and, dividing further by anticlinal walls, form short

* KARSTEN, 1893.
† STRASBURGER, 1879.
‡ LAND, 1907.
§ BOWER, 1882; LOTSY, 1899.
‖ KARSTEN, 1892, fig. 25; LOTSY, 1899.
¶ BOWER, 1882.

embryonic tubes, comparable to those of Welwitschia, though much less developed than these."* The cell-group referred to in this citation has, at the apex, a few cells whose form calls to mind the "initial," and, later, the "cap" cells of the Welwitschia pro-embryo, though one of them appeared to behave as an apical cell.† It is probable that a further study of more suitable material than was available‡ for Prof. BOWER'S investigation, will bring to light other points of resemblance in the structure of the embryos of Gnetum and Welwitschia.

The pro-embryo of Welwitschia has attained a high degree of specialisation as is seen in the apparently stereotyped structure of its secondary suspensor and in the marked regularity of the cell-divisions which occur in it. In these respects it may be compared with that of the higher Coniferæ, though traces of a parallel development are lacking. Indeed, the structure of the pro-embryo provides a strong argument in favour of placing Welwitschia and the Coniferæ on widely divergent lines of descent. If the embryonic plate is correctly identified, the pro-embryo of Welwitschia bears a striking resemblance to that of Araucaria.§ Whether this is limited to the cap and the embryonic plate or extends also to the many-celled suspensor is not clear, for the origin and structure of the latter organ are not known in sufficient detail in Araucaria. If this resemblance is not merely apparent, it is of great interest that a type of pro-embryo so different from that of other Gymnosperms should be common to genera so widely separated as Welwitschia and Araucaria. It must be regarded as an inheritance from an exceedingly remote common ancestry, and is no doubt correctly included among the many primitive characters which are retained by the modern representatives of the group Araucarieæ.‖

K. *The Endosperm after Fertilisation* (Plate 30, figs. 88 and 89).

The endosperm of a pollinated ovule in which fertilisation has not yet occurred is circular in transverse section and somewhat narrowed towards the antipodal end (fig. 89H). In unfertilised ovules little further growth takes place. A new phase is initiated soon after fertilisation. This secondary growth proceeds more rapidly after the penetration of the endosperm by the first pro-embryo. The terete form of the primary endosperm is soon lost and there results an organism elliptic in section, except at the antipodal end, where it remains terete; the major axis of the ellipse is tangential to the surface of the rachis of the cone; the endosperm thus acquires a persistent bilateral form which corresponds with that of other floral organs. The most advanced endosperm seen is about five times as broad as at the end of the period of primary growth (*cf.* fig. 89, H, K). Increase in length takes place mainly at the

* BOWER, 1882.

† BOWER, *loc. cit.*, figs. 6–8.

‡ BOWER, *loc. cit.*, p. 280.

§ STRASBURGER, 1879, Taf. 20, figs. 66–70; SEWARD and FORD, 1906, fig. 28, G, H.

‖ SEWARD and FORD, 1906, p. 395.

antipodal end, which for a time behaves as an organised growing point; the cells of a massive apical group are equally meristematic; its activity has greatly diminished by the time that the stage of fig. 89K is reached.* The maximum length found is three times as great as at the beginning of the secondary growth. There are also formed during this period two shoulder-like upgrowths, one on each side of the now empty bases of the embryo-sac-tubes (fig. 89, I, K). It may be that these are due indirectly to the absence of any opposing pressure in the empty cells of the fertile region which lies between them. The tissue of the endosperm in these later stages is not uniform in structure. If it contains one or more embryos, whether or not its upper tissues have been destroyed (see text-figure, *c*), an axial group of cells lying beneath the deepest embryo, and having the form of an inverted pyramid, is packed with starch (text-figure, *st.*), which at this time is not abundant elsewhere. Outside this storage region the cells differ considerably in size.† Very small cells usually form groups or bands irregularly distributed through a tissue mostly composed of much larger ones (fig. 88).

Canals formed by the growth of deep-seated embryo-sac-tubes (fig. 89H) are obliterated in the course of the secondary growth of the endosperm. Compartments whose nuclei remain free but do not produce tubes (*cf.* fig. 38B) are not found in an endosperm in which secondary growth is well advanced; they presumably collapse and their contents become absorbed.

Between the stage of fig. 89K and that of the adult seed the endosperm increases until all the nucellus, except a thin brown investing skin and the shrivelled remains of the nucellar cone, has disappeared. The adult seed thus has two distinct seed-coats, viz., the nucellar tegmen and the outer horny winged envelope formed by two opposite leaf structures. Owing to the intercalary growth of the nucellus above the origin of the outer envelope and the depth to which the interposed tissue is penetrated by the endosperm, the crushed integument covers little more than the shrivelled remains of the nucellar cone.

Summary and Discussion of Results.

Both male and female cones are frequently produced in great profusion.

The inflorescence is normally a compound dichasial cyme more or less modified by arrest. In some cases it consists of a single cone.

The branching of the inflorescence is carried further in the male than in the female.

Subfoliar inflorescences are produced with great frequency.

Macrospores and embryo-sacs are very frequently present in the pith region of the axis of the female cone. This implies that the ovule is cauline and confirms the view already based by most authors on other characters.

In the heterotypic division of the microspore-mother-cell the spindle is at first

* An antipodal meristematic cone seems to be organised also in Gnetum (LOTSY, 1903, Taf. 9, fig. 2).

† PEARSON, 1906, p. 293.

multipolar, later becoming bipolar. The post-meiotic number of chromosomes is between 22 and 26—probably 25.

The pollen-grains frequently germinate in the micropylar tube at a considerable distance above the top of the nucellus. In these cases the pollen-tube grows downwards in the fluid medium which fills the micropyle at the time of pollination.

A single macrospore-mother-cell is organised. A condition later than synapsis is the only stage of the meiotic divisions that has been found.

Only one macrospore is functional. Sometimes two, rarely three, pass through the early stages of germination. In these cases the lowest eventually develops at the expense of the rest.

In the development of the embryo-sac a large central vacuole is not formed; this may indicate that the growth of the cytoplasm and the multiplication of the nuclei are more rapid than is usual among the Gymnosperms. The nuclei divide simultaneously. The tendency to establish a polar arrangement of the nuclei which is manifested after the first mitosis in the macrospore is not seen in later stages.

After the last simultaneous division, the sac probably contains 1024 free nuclei which are equivalent in all visible characters.

A rapid increase in the length of the sac, occurring principally in its upper part, causes a more scattered distribution of the nuclei which are closely crowded after the last simultaneous division. As a result the nuclei of the upper fourth are more widely separated from one another than are those of the rest of the sac. The scattered nuclei of the upper fourth are sexually functional; the more crowded nuclei of the lower three-fourths, potentially equal to the rest, give rise to a sterile tissue, the endosperm.

The whole sac now becomes incompletely septate into compartments which are very irregular in form and size. Those of the upper fourth contain few nuclei, usually not more than six; the great majority of those of the lower three-fourths enclose many nuclei—commonly 12 or more; a few of the latter frequently resemble those of the upper fourth in that they contain only six nuclei or less.

The walls of compartments of the micropylar fourth grow upwards into the nucellus as tubes into which the nuclei and most of the cytoplasm pass. These are the embryo-sac-tubes; the nuclei within them are functional gametes.

The sexual nuclei lie in contact with one another in the young tubes. Rarely they fuse within the tube and form a fusion-nucleus quite similar to an original endosperm-nucleus. They are apparently quite free in the cytoplasm of the embryo-sac-tube; in some cases a thin layer of cytoplasm immediately surrounding the nucleus stains more deeply than the rest.

Fertilisation normally occurs in the lower half of the nucellar cone not far from the line B in the text-figure. Sometimes it is effected in the upper half of the nucellar cone. It probably rarely takes place below the level of the top of the embryo-sac. It never occurs within the embryo-sac.

In the lower three-fourths of the sac the free nuclei enclosed in a compartment rarely undergo division. When this does occur they divide simultaneously or, apparently, the division may be confined to a single nucleus. All the nuclei in a compartment which contains more than six fuse and it thus becomes a uninucleate cell.

Cells formed in this way constitute the primary endosperm. The single nucleus of each is formed by the fusion of potential gametes. This conclusion is based on the following evidence:—

1. At the stage of fig. 21 (the last simultaneous division of the free nuclei being completed), the nuclei are of the same generation—the eleventh from the macrospore-nucleus.
2. They are all alike in all visible characters.
3. Shortly before septation commences they show differences in size and staining capacity; but they all pass through the same series of changes and ultimately are again all alike. The observed differences are due to the fact that these changes commence at the micropylar end sooner than in the lower part of the sac.
4. When septation is completed some compartments contain few nuclei (six or less); others a larger number. The latter are normally confined to the lower three-fourths of the sac; the former may occur anywhere, though they usually fill the upper fourth of the sac and are either absent from or much less abundantly developed in the lower three-fourths.
5. In compartments containing less than six, wherever they may be situated, the nuclei do not fuse, but remain free; these are functional gametes. Fusion occurs only in those compartments in which the nuclei are in greater number; a sterile tissue is thus formed.
6. In an abnormal case in which the upward growth of the sac was impeded by a second one above it, compartments which normally would have contained gametes and produced embryo-sac-tubes became uninucleate endosperm cells by nuclear fusion.
7. Under certain circumstances no endosperm is formed at all, but, with the exception of a few which succumb to the greater activity of their neighbours, all the nuclei remain free and every compartment produces an embryo-sac-tube of the normal type.
8. In two cases (fig. 30, A and B) about six gametes in an advanced embryo-sac-tube have fused to form a nucleus in all respects like that of a primary endosperm-cell.
9. A small tissue found in an embryo-sac-tube and consisting of seven cells is interpreted as an endosperm formed by such a nucleus as those referred to in § 8.

The cells containing single fusion-nuclei, filling the whole or almost the whole of the lower three-fourths of the sac, form a nutritive tissue or organism—the endosperm —which undergoes considerable increase in size during two stages of growth, the one occurring before and the other after its penetration by the first pro-embryo.

This endosperm, resulting from the fusion of gametes, differs fundamentally from the prothallus of the lower Gymnosperms. It is here called a trophophyte.

On the germination of the pollen-grain the generative cell increases in size, becomes elongated, and moves down the tube behind the tube-nucleus. The nucleus of the generative cell divides once, giving two male nuclei.

The tube-nucleus usually shows signs of breaking down by the time that it reaches the level of the line B in the text-figure; it eventually disappears. In rare cases the pollen-tube passes down the nucellus to the chalazal region of the ovule.

The bi-nucleate generative cell frequently remains undivided; it may however become broken into two uninucleate portions.

The male nuclei may either remain spherical in form or become elongated. Both may persist until the time of fertilisation or one becomes disorganised.

When cytoplasmic communication is established between the pollen-tube and an embryo-sac-tube, the leading female nucleus enters the generative cell within which fertilisation occurs. A resting fusion-nucleus is formed as a result of fertilisation; the whole or part only of the cytoplasm of the generative cell, with perhaps a small contribution from the embryo-sac-tube, forms the cytoplasm of the oospore. Several oospores are commonly formed in the same nucellus.

The oospore elongates, especially in the direction of the embryo-sac. The nucleus moves towards the lower end of the pro-embryonal tube thus formed, and divides. In rare cases the pro-embryonal tube grows towards the micropyle.

The division of the primary pro-embryonal nucleus is followed by the formation of a wall cutting off a small cell at its lower end. This wall apparently arises as a centripetal ingrowth from the wall of the tube.

The upper cell undergoes no further division. It increases greatly in length, at first as the primary suspensor and later as the axial cell of the secondary suspensor.

From the lower cell is produced a group of initial cells, from which are derived successively: (*a*) 8 inner cortical cells; (*b*) 16 outer cortical cells of the secondary suspensor; (*c*) a presumed "embryonic plate" of 8 cells; (*d*) a ring of 16 cells around and slightly below the latter; (*e*) a terminal "cap" of 8 cells.

By the time that the series (*b*) is formed, the tip of the pro-embryo has usually reached the endosperm within which its further development proceeds.

It is possible that the later stages of the development of the embryo are passed through after the seed has become detached. Secondary growth in the endosperm, which commences after fertilisation, is to some extent localised in the antipodal end. Finally, all the nucellus is displaced save a thin brown tegmen and the shrivelled remains of the cone. The adult endosperm has a bilateral symmetry.

The trophophyte of Welwitschia is morphologically so different from the prothallus of Ephedra that these genera must be placed in two distinct groups, whose affinities are indicated by characters of subordinate importance. What little is known of the development of the endosperm of Gnetum leads to the conclusion that it is a trophophyte homologous with that of Welwitschia. Since this conclusion accords with other evidence of a fairly close degree of affinity between these genera, they are placed together in the following scheme of subdivision of the group Gnetales.

Group I. Ephedroideæ.—Endosperm a true prothallus bearing archegonia; microspore on germination producing one or two prothallial cells, a tube-nucleus, a wall-(stalk-) nucleus and two gametes; oospore-nucleus by successive divisions giving rise to a small number of free nuclei (frequently eight), each of which forms a pro-embryo; complete intraseminal development of the embryo attained before the seed falls.

Group II. Gnetoideæ.—Female gametophyte reduced to a plasmodium containing free sexual nuclei which are very numerous; endosperm formed as a result of gamete-fusion; microspore, on germination, producing an evanescent prothallial nucleus, a tube-nucleus and two gametes; oospore giving rise directly to a single pro-embryo;* intraseminal embryo-development, or only the later stages of it, probably deferred until the seed has fallen.

Gnetum.—The embryo-sac or only the fertile end of it unseptate until after fertilisation; primary endosperm formed after or before fertilisation; fertilisation occurring within the embryo-sac which is penetrated by the pollen-tube.

Welwitschia.—Whole sac incompletely septate and the primary endosperm formed before fertilisation; female gametes carried by the embryo-sac-tubes into the nucellar cone, where fertilisation takes place.

The absence of the vegetative part of the gametophyte as well as of the sterile tissue of the archegonium, both of which are present in Ephedra, does not admit of explanation. It is not improbable that a clue to the solution of this problem may yet be found in some species of Ephedra or of Gnetum whose life-histories are at present unknown. It is indeed possible that the fusion believed to occur among the nuclei of the jacket-cells of *Ephedra distachya* represents one of a series of changes which has led to the conditions now obtaining in Welwitschia. A detailed study of every available form of both genera is certainly called for. In the absence of any direct evidence as to the manner in which the free gametes of the Gnetoideæ have been derived from the intrasporic prothallus and archegonia—and this must be assumed to have occurred—two alternative suggestions are tentatively advanced.

* In *G. Gnemon*, etc.; in some other species, according to KARSTEN, the oospore-nucleus behaves somewhat as in Ephedra.

There is a well-marked tendency towards the elimination of wall-tissue in the archegonium of the higher groups. This is carried so far that in the Cycads and some Conifers the wall is reduced to two neck-cells. If the reduction proceeded but little further along the same lines, an archegonium, represented merely by an oosphere and a ventral canal-cell, would result. Such archegonia occur not infrequently in *Picea excelsa*.* The ventral canal-cell is almost certainly to be regarded as an arrested gamete.† If the complete reduction of wall-tissue were accompanied by the reversion of the ventral canal-cell to its presumed primitive condition of a functional gamete, the archegonium of the lower gymnosperm would be reduced to two free gametes.

Although in most of the living archegoniate Spermaphytes, so far as they have been investigated, the limited number and definite localisation of the archegonia indicate a high degree of specialisation, cases are known in which neither of these conditions is realised. Perhaps the most remarkable of these is found in *Microcycas calocoma*,‡ in which the archegonia are very numerous and the irregularity of their distribution so marked that they are not even confined to the surface of the prothallus. This is regarded by CALDWELL as a primitive character. A similar tendency to increase the number of archegonia appears in Araucaria,§ Agathis§—in which as many as 60 may be organised in a prothallus—and in Sequoia.‖ These cases also the authors are inclined to consider as primitive. But, on the other hand, so marked an irregularity in the distribution of the archegonia as is seen in these forms is not characteristic of the lower Archegoniatæ, where, however, their usually limited numbers and more or less definite localisation are no doubt largely determined by physiological conditions connected with fertilisation and, more especially, with the nutrition of the embryo. On the evidence at present available, it is at least a possibility that, as LAND¶ has suggested, this tendency to increase the number of archegonia, instead of being a relic of primitive conditions, represents an advance towards Gnetoid conditions.

If the number of archegonial initials were increased to the utmost extent, there might result a sac in which each of the free nuclei present after the last simultaneous mitosis which precedes septation would represent an archegonial initial. If further the formation of archegonial wall-tissue were completely suppressed and the ventral canal-nucleus reverted to its primitive condition of sexuality, all the nuclei in the adult sac would be free gametes.

It is therefore of some interest to find that after the completion of the last

* MIYAKE, 1903, p. 364, figs. 60 and 61.

† BLACKMAN, 1898; COULTER and CHAMBERLAIN, 1901, pp. 86, 87, fig. 65; LYON, F., 1904; DAVIS, 1903, 1904; HOLFERTY, 1904.

‡ CALDWELL, 1907.

§ SEWARD and FORD, 1906, pp. 366, 367.

‖ LAWSON, 1904.

¶ LAND, 1907.

division of free nuclei in Welwitschia the number present is greater than that recorded for some other Gymnosperms. In Ginkgo,* Taxus,† *Ephedra helvetica*,‡ and *E. trifurca*,§ the total number of nuclei present in the sac in the stage immediately preceding septation is stated to be about 256. If there has been no departure from the simultaneous character of the nuclear divisions, all these 256 nuclei are of the same generation—the ninth in a direct line from the nucleus of the macrospore. There are, however, several cases known in which the number at a corresponding stage is much larger, *e.g.*, in *Pinus Strobus* "about 2000,"‖ in *Sequoia sempervirens* over 500,¶ and in *Ephedra distachya* "about 1000."** In Welwitschia, assuming that the mitoses are simultaneous throughout, the actual number is 1024. All these nuclei which must now be regarded as potentially sexual belong to the eleventh generation from the macrospore nucleus. It is stated that in Ephedra the first division of a primary alveolus which gives rise to an archegonium results in the formation of the central cell and primary neck cells,†† but since this observation possibly refers to *E. distachya* only, it cannot at present be interpreted to mean that the nuclei of the Welwitschia-sac are of the same generation as the oosphere of *E. helvetica* or of *E. trifurca*. But it may be suggested that the large number of nuclei produced in the Welwitschia-sac is perhaps connected with the elimination of the prothallus and archegonium as such and their substitution by free gametes.

An alternative suggestion may be based upon the behaviour of vegetative nuclei in the prothalli of certain so-called "apogametic" ferns.‡‡ Here the normal sexual act is replaced by the fusion of two nuclei of adjacent cells of the prothallus; the cells whose nuclei behave thus may be situated anywhere in the younger parts of the plant. Whatever may be the precise difference between the normally vegetative and the sexual nuclei of the prothallus, it may thus disappear to the extent of enabling the fusion of two belonging to the former category to produce a normal embryo. It is possible that the development of the characters of the female gamete in all the nuclei of the prothallus, together with the suppression of wall-formation, has been concerned in bringing about the change from the conditions of the lower seed-bearing plants to those of the Gnetoideæ.

The early stages of development of the Welwitschia-sac correspond very closely with those described for the lower Gymnosperms, and the changes which are involved

* Hirasé, 1895.
† Jaeger, 1899, p. 252.
‡ Jaccard, 1894, p. 16.
§ Land, 1904, p. 10.
‖ Ferguson, 1904, p. 84.
¶ Lawson, 1904, p. 12.
** Berridge and Sanday, 1907, p. 129.
†† Berridge, 1907, p. 281.
‡‡ Farmer and Digby, 1907, p. 175.

in the acquisition of sexual characters by all the sac-nuclei do not manifestly occur earlier than the end of the free-nuclear condition. In Gnetum all the nuclei of this last stage are apparently already potentially sexual. In Welwitschia they undergo certain changes in form and staining capacity in later development and it may be that here sexuality is not acquired until after septation. But in both, the gametes are free nuclei irregularly distributed in a non-septate or incompletely septate plasmodium. It is therefore probable that in the transition, by a gradual process or *per saltum*, from the sac of the lower Gymnosperm to that of the Gnetoideæ, the nuclei which became endowed with sexuality were those which filled the still unseptate sac at the end of simultaneous nuclear division. Hence the gametes in the primitive sac of the Gnetoid type were free nuclei. The view that the Angiosperm-sac is a derivative from the same type is advanced below. One of the signs of its higher degree of specialisation is the fact that its gametes, functional and reduced, are no longer free nuclei but cells.

It remains to be considered whether the Gnetoid endosperm is unique or whether, on the other hand, it may not be regarded as representing a primitive condition, from which that of the higher seed-bearing plants has been evolved. The former is the product of the division of many fusion-nuclei, the latter of one; in both the fusion-nucleus results from the union of nuclei of the same generation as the functional gametes. Angiosperms are described in which the endosperm is apparently formed without antecedent nuclear fusion; these seem to be exceptional and rare.

The sac of Peperomia exhibits certain peculiarities, on account of which a comparison between it and that of Gnetum has already been suggested.* In the four-nucleate stage of *P. pellucida* the sac shows no polarity, but the nuclei are arranged peripherally, "like the spores of a tetrad"†—in fact, precisely as in Welwitschia in the corresponding stage (fig. 16). When the mitoses are completed there are sixteen nuclei in the sac belonging to the fifth generation from the macrospore. These are "rather evenly distributed through the sac"; one of them increases in size and becomes the nucleus of the oosphere; of the rest, eight fuse to form the primary endosperm-nucleus. Each successive division of this nucleus and its descendants is followed by the formation of cell-walls,‡ as in Welwitschia. In *P. hispidula*, of the sixteen sac-nuclei all but two fuse to form the definitive nucleus.§ The general resemblance of this process to that by which the Welwitschia endosperm is formed is very striking; in both cases the constituents of the primary endosperm nuclei are of the same generation as the functional gametes; in Welwitschia, not only is this the case, but the constituents of the fusion-nuclei are themselves potential gametes. There is no evidence that the fusing nuclei of

* COULTER and CHAMBERLAIN, 1903, p. 90.

† The same arrangement is recorded for the Penæaceæ (STEPHENS, 1908).

‡ JOHNSON, 1900; CAMPBELL, 1901.

§ JOHNSON, 1907, *ex* SARGANT, 1908.

Peperomia are potential gametes, but they are probably to be regarded as the arrested representatives of nuclei which in earlier forms were potentially sexual.

CAMPBELL* regards the sac of Peperomia as primitive among Angiosperms. JOHNSON,† on the other hand, inclines to the view that its abnormalities rather indicate a higher degree of specialisation. As CAMPBELL has pointed out, the evidence for this view is inadequate. Even if the plant is reduced in its vegetative parts and in its floral structure, and if, as ARBER and PARKIN‡ maintain, the crowding of the flowers into a spike is not a primitive feature, it does not follow that a departure from the "normal" structure in the embryo-sac is not primitive. The doubled number of nuclei cannot be attributed to reduction, and neither this nor the absence of polarity is in agreement with the suggestion of increased specialisation. In Cypripedium,§ in which there is good reason to believe that a high degree of specialisation has been attained, the number of sac-nuclei is just half that found in normal cases. While irregular divisions among the sac-nuclei might conceivably result from disturbing conditions in the environment, it is difficult to suppose that any factor other than heredity is concerned in the regular production of twice the normal number of sac-nuclei and the organisation of the oosphere in a generation once removed from that of the oosphere in the normal Angiosperm. CAMPBELL'S view further receives strong support from the facts now established for Welwitschia. It is a justifiable conclusion, from the evidence available, that the sixteen-nucleate sac of Peperomia represents a stage intermediate between an ancestral type, with a massive primary endosperm, and the more recent eight-nucleate form, which is found in the majority of the higher Angiosperms. The fact that the number of constituents of the fusion-nucleus varies in different species of Peperomia is favourable to this view, for in Welwitschia, a form less specialised, in which there are many fusion-nuclei in the same sac, the number of their constituents shows a wide range of differences.

In this connection, NAWASCHIN'S observations on *Juglans regia*‖ are of great interest. There is no organised egg-apparatus, but the sac contains several free nuclei, any one of which may be fertilised. KARSTEN‖ has extended this observation to other species of Juglans. Except for the small number of nuclei, this seems not far removed from the condition of the Welwitschia-sac. In *Aglaonema commutatum* "multiple nuclear fusion" frequently occurs, and it is likely that it gives rise to the primary endosperm-nucleus.¶

In the higher Angiosperms, in which specialisation of the gametophyte has proceeded

* CAMPBELL, 1901.
† JOHNSON, 1900.
‡ ARBER and PARKIN, 1907.
§ PACE, 1907.
‖ COULTER and CHAMBERLAIN, 1903, pp. 90, 91.
¶ CAMPBELL, 1903, p. 674.

further, and the oosphere appears in the fourth or third generation, the fusion-nucleus contains only two constituents. These are normally the two polar nuclei. But this is not constantly the case, for in Cypripedium, in which the sac contains only four nuclei, the constituents of the primary endosperm-nucleus, in addition to a male nucleus, are derived, one from a synergid and the other from the antipodal region.* Of the six nuclei of the normal sac which do not participate in the fusion, one is functionally sexual; two others, those of the synergids, are in some cases fertilised and produce normal embryos.† Embryos, said to be apogametic, are described as originating from the antipodal cells,‡ from the upper polar nucleus,§ and from the cells of the endosperm,‖ the last in *Balanophora elongata*, in which the primary endosperm is not formed from a fusion-nucleus. An "antipodal egg" resulting from the fertilisation of an antipodal nucleus by a sperm is believed to occur in some species of aster.¶ Clearly, then, the synergid-nuclei sometimes are potentially sexual, and although no cases in which the polar or antipodal nuclei become normally fertilised are certainly known, it is probable that as regards sexuality they are merely more reduced than the synergid-nuclei.

The group of three or more antipodal cells in the angiosperm-sac has acquired an absorptive function in many families, but in a great number of others they appear to play very little if any part in the activities of the sac, but become disorganised and are eventually replaced by endosperm. It is interesting to compare this behaviour with that of the "retarded prothallium cells" of the fertile part of the sac of *Gnetum Gnemon*.** The nuclei of these cells are related to the functional gametes in the same way as are the unfused nuclei of deep-seated compartments in the Welwitschia endosperm (fig. 38B); and since they presumably belong to the same generation as the sexual nuclei, they are comparable with the antipodal and synergid-nuclei of the normal Angiosperm and with the "accessory" nuclei of Peperomia,†† though physiologically they probably differ in that they normally retain a potential sexuality. And their fate is the same as that of the non-persistent antipodal cells, the synergids and the accessory nuclei—after fertilisation "the fertile part of the embryo-sac . . . is gradually obliterated, owing to the growth of the sterile part; its retarded prothallium cells play no *rôle* whatever."** In both cases the endosperm derived from fusion-nuclei destroys and replaces all the contents of the embryo-sac, save the embryos themselves.

* PACE, 1907.

† DODEL, 1891, *ex* COULTER and CHAMBERLAIN, 1903, p. 217; OVERTON, 1891, *ex* MANN, 1892, p. 373; GUIGNARD, 1901, *ex* COULTER and CHAMBERLAIN, 1903, pp. 170, 216, and *sqq.*, fig. 103.

‡ TRETJAKOW, 1895; HEGELMAIER, 1878, *ex* COULTER and CHAMBERLAIN, *loc. cit.*, p. 221.

§ TREUB, 1898; LOTSY, 1899, *ex* COULTER and CHAMBERLAIN, 1903, p. 218.

‖ TREUB, *loc. cit.*

¶ OPPERMAN, M., 1904.

** LOTSY, 1899, pp. 97, 98.

†† CAMPBELL, 1901, p. 110.

These considerations suggest that the embryo-sac of the higher Angiosperm is a highly specialised structure, derived through such forms as are represented by Peperomia, Juglans, and Penæa from ancestors in which the conditions of the Gnetoid sac were more or less nearly realised. If so, the endosperm of the Angiosperm was originally a trophophyte homologous with that of Welwitschia. In the course of the evolution of the existing Angiosperms it has become reduced, *pari passu*, with the appearance of the functional gamete in successively earlier generations, and the consequent reduction of the number of nuclei available for fusion. Its constitution has further been fundamentally modified by the inclusion of a male gamete in the primary fusion-nucleus from which it is derived.

There seem to be no cases described in which more than one primary endosperm nucleus is formed in the Angiosperm, while in the Gnetoideæ the number is large and the fusion is preceded by septation. This difference is no doubt closely connected with the great reduction in the number of sac-nuclei. In Corydalis a septation followed by fusion apparently very similar to that found in the Gnetoideæ is described. Whether the fusing nuclei in this case are the descendants of a normal definitive (fused) nucleus or of the two free polar nuclei does not seem to be certainly established.* In the Gnetoideæ the division of the fusion-nucleus is immediately followed by wall-formation. In the Angiosperm, on the other hand, the formation of a number of free nuclei usually precedes septation. In many cases, however, the first division of the definitive nucleus is followed by the appearance of a wall dividing the sac into two cells, and even when this does not occur a transitory cell plate is frequently formed.* Therefore it is probable that in the ancestors of the Angiosperms the successive divisions of the definitive nucleus and its descendants were followed by the formation of cell-walls as still occurs in Peperomia and in the Gnetoideæ.

One of the most marked features of the sac of the Angiosperm is its pronounced polarity. The difference between this condition and that of Welwitschia is perhaps not so great as would appear at first sight. In the latter, as also in Gnetum,† there is an inherent tendency towards a polar arrangement of its nuclei after the division of the macrospore-nucleus (fig. 15). But if corresponding stages of development up to the time of fertilisation are compared, the sac of Welwitschia is probably not much larger than that of the average Angiosperm, while the number of nuclei rapidly becomes much greater. It is therefore possible that the approximately uniform distribution of the nuclei in the former is connected with, perhaps a direct consequence of, the fact that after the first few nuclear divisions the sac is not large enough to allow of any other arrangement.

It has been seen that nuclei in any part of the Welwitschia-sac may remain unfused and so retain their sexuality. If this occurs more frequently in one part of the normally sterile region than in another it is, perhaps, at the antipodal end. Since,

* STRASBURGER, 1880.

† STRASBURGER, 1879, Taf. 14, fig. 60; KARSTEN, 1892, p. 210, fig. 5; 1893, Taf. 9, fig. 44.

however, this is not of general occurrence, it is probably not connected with the tendency to establish polarity seen in the earlier stage, but is rather due to the fact that growth of the sac is not uniform. The exceptional occurrence of a sac in which all the nuclei retain their sexuality and no endosperm is formed rather indicates that Welwitschia is descended from forms in which all the nuclei were potential gametes at the time of fertilisation, and the conditions were such that any one of them, wherever situated, might be fertilised. Theoretically these conditions are realised in the species of Gnetum studied by KARSTEN.

The lack of polarity in the embryo-sacs of Peperomia, Juglans, and other Angiosperms* in which there are retained other characters reasonably regarded as primitive, justifies the belief that the more typical angiosperm-sac has been derived from a form in which the polar arrangement did not persist beyond the bi-nucleate stage. A considerable reduction in the number of nuclei formed—owing to the organisation of the functional gametes in an earlier generation than in Welwitschia or Gnetum—especially if accompanied by an increase in the size of the sac, would be likely to result in a grouping of the nuclei rather than in their distribution in the cytoplasm. If this grouping commenced immediately after the first division of the macrospore-nucleus the result would be a bipolar arrangement such as is seen in the normal Angiosperm. If it were deferred until the appearance of the grand-daughter nuclei, there might be formed four peripheral groups such as are described for the Penæaceæ.† A group of three organised cells, originally potential gametes, would provide the materials for the evolution of the highly specialised egg-apparatus which is formed normally at the micropylar pole and, in some forms, is perhaps still present at the antipodal end. STEPHENS has suggested that each of the four groups of the Penæaceæ is a functional egg-apparatus.† If this is confirmed it will constitute important evidence in favour of regarding the antipodal group of the normal sac as a reduced egg-apparatus. But on the hypothesis now advanced this does not appear to be necessary in all cases. All that is assumed is that the eight nuclei are either all potentially sexual or, in most cases, seven or less are the reduced representatives of nuclei which possessed this character. And it is probable that in very many groups no such specialisation occurred in the antipodal cells as is characteristic of the functional egg-apparatus. Their persistence in the higher Angiosperms is no doubt largely to be accounted for by the fact that they have acquired a new function and play a more or less important part in the nutrition of the sac. In the remarkably reduced sac of Cypripedium an antipodal group is not formed.‡ Whether the organisation of antipodal cells in the Angiosperms has any connection with a chalazogamous method of fertilisation is doubtful. The fact that in Welwitschia the generative cell may be carried down into the tissue of the elongated internode below the origin of the

* COULTER and CHAMBERLAIN, 1903, p. 90; CAMPBELL, 1903, p, 670.

† STEPHENS, 1908.

‡ PACE, 1907.

integument, and therefore below the antipodal end of the sac, does not necessarily indicate the former occurrence of a specialised method of fertilisation akin to the chalazogamy of some Angiosperms; it may equally be a relic from a time when, the sac at the time of fertilisation being filled with equivalent gametes, the pollen-tubes entered it indifferently at any points in its wall. A further possibility is that the solitary case that has been seen is purely accidental and due to the unusual circumstance that the pollen-tube has evaded the embryo-sac-tubes in the nucellar cone and therefore continued its downward course.

The phenomenon of triple fusion, now known to occur in 52 genera and 22 families of Angiosperms,* has made more complex the problem of the morphology of the endosperm in this group. Diverse views as to the nature of the fusion and its phylogenetic significance have been advanced.† None of these is altogether in accord with the hypothesis that the endosperm of the primitive Angiosperm was essentially homologous with that now described for Welwitschia. No process that can possibly be considered as phylogenetically connected with the triple fusion occurs in Welwitschia. There is at present no evidence to show that a male gamete plays any part in the formation of the primary endosperm-nucleus in Peperomia or in any other Angiosperm in which more than two of the sac-nuclei are concerned therein. It is therefore suggested that the male nucleus was not included in the fusion which gave rise to the definitive nucleus in the primitive Angiosperms, and that the origin of the phenomenon is not to be sought in the higher Gymnosperms. It is rather probable that triple fusion was called into existence by the continued reduction of the number of sac-nuclei and consequently of the number available as constituents of the primary endosperm-nucleus.

It may be assumed that the movement of the polar-nuclei towards one another or of the lower one towards the upper is brought about by a mutual attraction, no doubt of a chemiotactic nature. In some species of Alchemilla‡ this attraction is sufficient to draw together not only the polar-nuclei but, with them, those of the antipodal cells and synergidæ. The second male nucleus, after its projection into the embryo-sac, is free to move, and, according to STRASBURGER'S observations on Monotropa,§ is carried down to the polar-nuclei by the streaming movements in an axial strand of cytoplasm. It is probable that its movement is affected by the same directive force which is potent in the case of the polar-nuclei. In some cases there is a third possibility, viz., that the male nucleus may possess some power of independent motion which enables it to make its own way to the fusing group,‖ perhaps also under the influence of a directive force. But whether the male nucleus retains the motility as well as the

* SARGANT, 1908, p. 126.

† SARGANT, 1900; LAND, 1907; THOMAS, 1907; BERRIDGE, 1907.

‡ MURBECK, 1902, *ex* COULTER and CHAMBERLAIN, 1903, p. 93.

§ STRASBURGER, 1900, *ex* SARGANT, 1900.

‖ SARGANT, *loc. cit.*

form of the antherozoid or, having lost both, is carried by the streaming cytoplasm, with or without the influence of an internuclear attractive force, the conditions prevailing in the angiosperm-sac are highly favourable to the occurrence of the triple fusion. It is suggestive that when, as sometimes happens, more than one of the sac-nuclei retain their sexuality and therefore presumably exert a more powerful attractive influence upon the second male gamete, the triple fusion is prevented and a true double fertilisation occurs; this occurs, *e.g.*, in *Naias major*, where one male nucleus fertilises the oosphere and the second may fuse with a synergid, in which case two normal embryos are formed.* It is therefore worthy of consideration whether the triple fusion is not primarily a result of the peculiar conditions of the higher types of angiosperm-sac and confined to sacs in which they are realised.

Three suggestions have been made as to the physiological importance of the triple fusion. The stimulus to renewed growth is increased,† and the introduction of a set of characters from the individual which stands as the male parent of the embryo may raise the nutritive efficiency of the resulting endosperm.‡ Further it is supposed that the increase in the number of chromosomes caused by the entrance of the male nucleus may serve to secure the degeneracy of the resulting tissue.§ In Welwitschia the primary fusion-nuclei are numerous. If all but one were eliminated this would closely resemble in origin the definitive nucleus of Peperomia, and *ceteris paribus* would produce a very small mass of endosperm tissue. Miss SARGANT points out that a similar result is achieved by the definitive nucleus of Peperomia.‖ In this case there is no evidence that the triple fusion occurs, though the difficulties of observation are great and it appears to be still possible that it may have been overlooked. If, however, the origin of the definitive nucleus is correctly described, Peperomia affords a most interesting comparison with Welwitschia on the one hand and with the higher Angiosperm on the other—it suggests a *raison d'être* for the triple fusion. With the reduction in the number of the sac-nuclei, and consequently of the number of fusion-nuclei, the trophophyte as established in Welwitschia is no longer an efficient endosperm. Its efficiency is increased by the incorporation in the fusion-nucleus of a male gamete. It is conceivable that the introduction of this new factor has not only saved from extinction a race which discarded the prothallus in favour of the trophophyte, but has also been of fundamental importance in determining the success of the Angiosperms in the present epoch.

The change from the intrasporic female prothallus retaining the sexual apparatus of the Pteridophyte to the trophophyte with free gametes, judged by its results, has been of hardly less importance in the evolution of the higher plants than was the

* GUIGNARD, 1901, *ex* COULTER and CHAMBERLAIN, 1903, p. 216, fig. 103.

† SARGANT, 1900, p. 705.

‡ THOMAS, 1907.

§ SARGANT, 1900.

‖ SARGANT, 1908, p. 129.

earlier advance from the conditions of the holophytic or saprophytic prothallus to those of the seed. The latter has already commenced in the living Pteridophytes; there is at present no evidence as to the manner in which the former was initiated. The conclusion that the intrasporic prothallus underwent a series of reductions in the evolution of the higher archegoniate Gymnosperms is not justified by such evidence as is at hand. But it is possible that its replacement by the trophophyte was connected with the development of a necessity for a more active and efficient means of nourishing the embryo than it provided. Such a necessity might arise through the shortening of the period comprised between pollination and the maturation of the seed. It is probable that the seed appeared independently in different groups of heterosporous Pteridophytes, and it is equally likely that, under stress of similar conditions, more than one group of Gymnosperms discarded the prothallus for a nutritive organism originating in gamete-fusion—a possibility which must be considered in discussing the relationships between the Gnetoideæ and the Angiosperms. After the establishment of the trophophyte it seems that reduction and specialisation set in. The oosphere was organised in successively earlier generations, and the number of nuclei available for fusion became successively smaller. From a condition in which many fusion-nuclei were formed (as in Welwitschia) there was derived a sac—such perhaps as is now seen in Peperomia—in which only one was produced. The endosperm resulting is apparently no longer an efficient organism. Still further reductions in the direction of the conditions of the higher Angiosperms may be supposed to have involved a further lowering of the efficiency of the endosperm until the triple fusion appeared.

The direct descent of the Angiosperms from the Gnetales has been much discussed, and although Lotsy and other authorities have pronounced against it, it still finds a place among current theories. If the essential homology of the endosperm in the former group with that of the Gnetoideæ be admitted, there is established between them a closer degree of affinity than has hitherto been suspected. The absence of archegonia and the freedom of the female gametes within the sac, together with the total disappearance of the vegetative portion of the gametophyte, are no less significant of the existence of a line of separation between the lower Gymnosperms (including Ephedra) on the one hand and the Gnetoideæ and the Angiosperms on the other. The reduction of the male gametophyte and sexual cells in Gnetum and Welwitschia to conditions almost identical with those prevailing in some Angiosperms and the small size of the male cells in Welwitschia add further emphasis to this distinction. Some small differences in the development of the microsporangium and in the dehiscence of the pollen-grain* are not likely to be of primary importance. The relations of the floral structures in the two groups have already been discussed, and these seem to place considerable difficulty in the way of regarding any Angiosperm as directly descended from Welwitschia and perhaps equally from Gnetum. The living Gnetales form a group of undoubted antiquity.

* Pearson, 1906.

If the endosperm of the Angiosperm is morphologically of the same nature as that of Gnetum and Welwitschia, it must follow that, in a broad sense, the Angiosperms and the Gnetoideæ are derived from the same stock.* It is, however, improbable that the two groups are on the same line of descent. Gnetum and Welwitschia are rather the last representatives of a race which separated from the main Angiosperm line after the replacement of the intrasporic prothallus by the trophophyte.

An attempt has been made to show that the floral structures of Welwitschia may conceivably be derived from a type of strobilus similar to that which was characteristic of Bennettites. Very little is known about the ovule of this extinct type and, indeed, it is not certain that unfertilised ovules have yet been found. But certain characters of ovules which do not contain the normal dicotyledonous embryo† are suggestive of a closer affinity with Welwitschia than any that can be deduced from a possible resemblance of external floral characters. The evidence is however at present quite inadequate and the following comparison is purely tentative :—On WIELAND'S Plate 30 a section is represented passing through two ovules in which the cavity enclosed by the "thin-walled nucellus" is nearly or entirely filled by "large, rounded, and thin-walled cells." This tissue is "irregularly, though characteristically, traversed by bands, which under very favourable conditions are seen to be made up of small cells."‡ The author does not regard this tissue as a prothallus, because there is no indication of the presence of archegonia. He inclines rather to the opinion that it is a massive undifferentiated embryo such as that of Ginkgo. Against this it may be urged that in its comparatively undifferentiated condition§ the embryo of Ginkgo, while it fills the cavity of the archegonium, has a transverse diameter much smaller than that of the prothallus; in a transverse section it would not "nearly, if not entirely, fill the seed cavity." And further, a section of the embryo of the living Ginkgo is not traversed by irregular bands of small-celled tissue.|| There is, of course, also the possibility that the sections, being transverse, have passed through the prothallus below the level of the archegonia. An alternative suggestion is that these ovules are, as WIELAND at first suggests, unfertilised or contain only undeveloped pro-embryos and that the tissue in question is not a prothallus but a trophophyte of the same character as that which occurs in Welwitschia. This would account for the absence of archegonia and also for the presence of the irregular bands of small-celled tissue which is a constant character of the Welwitschia endosperm (fig. 88). If this is the true nature of the sterile tissue of the Bennettitean sac, the possibility of a phylogenetic connection between Bennettites and Welwitschia, which has already been discussed, will be very greatly increased.

* *Cf.* COULTER and CHAMBERLAIN, 1903, pp. 286, 287.

† WIELAND, 1906, pp. 124, 125. ‡ *Loc. cit.*

§ COULTER and CHAMBERLAIN, 1901, fig. 35; LYON, H. L., 1904, Plate 33, fig. 8; Plates 30, 32.

|| *Loc. cit.*

Perhaps the most astonishing feature of the life-history of Welwitschia is the acquisition by the apparatus connected with the female gametes of a behaviour which is so constantly characteristic of the male. The cells containing the female gametes not only have departed from the state of quiescence which marks the female organ in all but the lower Algæ and Fungi, but, in the entrance of the female nucleus into the male cell and in the very large part played by its cytoplasm in the construction of the oospore, we have characters which are probably unparalleled in the vegetable kingdom. There is an important difference between the formation of haustorial tubes by the micropylar end of the sac in Gnetum* and in some Angiosperms† and the growth of the embryo-sac-tubes in Welwitschia. In the latter the tubes are formed by the membranes of the compartments containing the fertile nuclei, while in the former the nucellus is penetrated by the wall of the embryo-sac itself. In no species of Gnetum yet described are there any structures of like character.‡ The apex of the archegonium of *Ephedra distachya* grows upwards into the neck as the time for fertilisation approaches and may pass through it into the pollen-chamber.§ A growth of a similar kind is recorded for *Cephalotaxus Fortunei*.‖ While there is a certain similarity between this and the growth of the embryo-sac-tubes of Welwitschia, it can hardly be supposed that they are phylogenetically connected. At the same time the high degree of organisation to which the latter have attained makes it improbable that they have originated *de novo* in Welwitschia. Since they invariably attain nearly to that region of the nucellar cap in which fertilisation normally occurs before the ovule is pollinated, it seems probable that their importance in the economy of the plant is primarily associated with a shortening of the period between pollination and fertilisation—in other words, that they are of the nature of an adaptation to extreme xerophytic conditions. This was probably the view entertained by STRASBURGER when he wrote of them: "doch scheint mir dies eine specielle Anpassung zu sein, auf die kein zu grosses Gewicht zu legen ist."¶ When these words were written it was not known that the formation of the tubes was preceded by a partial septation of the fertile end of the sac. A similar septation of the lower part of the sac normally occurs in Gnetum, usually after fertilisation. In *G. Gnemon*, however, the process is advanced a stage and precedes fertilisation. In Welwitschia it has been extended from one end of the sac to the other. Even if the tubes have arisen in the manner suggested it is impossible that the antecedent septation can have originated merely as an adaptation to external conditions.

* KARSTEN, 1893; PEARSON, 1906, p. 288.

† HOOKER, 1863, p. 39.

‡ The curious tubular cells in the upper part of the sac of *Gnetum Ula* (LOTSY, 1903, Taf. 9, figs. 2, 3, 4; Taf. 10, fig. 1), which Dr. LOTSY believes to be parthenogenetic embryos, are very suggestive of incipient embryo-sac-tubes.

§ BERRIDGE and SANDAY, 1907, p. 132.

‖ COKER, 1907, fig. 7.

¶ STRASBURGER, 1872, p. 295.

The separation of Welwitschia from the immediate neighbourhood of Ephedra and the establishment of affinities, real if not close, with Gnetum, might be anticipated from the geographical distribution of the three genera. Ephedra is not represented in Africa, south of the Mediterranean region. Its absence from Equatorial and Southern Africa is very striking in view of its wide east and west distribution in the warm temperate regions of the Northern Hemisphere and its development on both sides of the Equator in America. Gnetum, with an equally extensive east and west range in the Tropics, is represented in Africa by two climbing species confined to the Guinea region, viz., *G. Bucholzianum* in the Cameroons* and *G. Africanum* on the Quetta Mountains,† about 120 miles from the coast at St. Paul de Loanda. The littoral strip of desert, of whose flora Welwitschia forms the most striking feature, is a continuation northwards of the arid belt which fringes the western coast of extratropical South Africa.‡ On the north and north-east it is bounded by the southern extension of the Guinea region and on the east by the woodland formation§ of the mountainous districts of inner Damaraland. It has been shown§ that, in spite of the remarkable development of endemic and peculiar forms in the desert strip, there are, nevertheless, indications that its flora is allied to that of the Acacia region, which itself is probably connected with that of Guinea through Ovamboland and the lower and intermediate slopes of the Angola Plateau. The geographical evidence is therefore not opposed to the view that the floras to which the African Gneta and Welwitschia respectively belong are descended from the same phytogeographical stock. At the same time, the physical geography of the coastal area of Damaraland,‖ together with the unique characters of the dominant elements of its flora, indicate that the conditions which have led to its differentiation have persisted through an enormously long period. The fact that Welwitschia diverges so widely in its external characters, as probably also in the details of its reproduction, from species which, for geographical reasons, may be supposed to be more nearly related to it than any other living plants, is therefore not remarkable. If forms intermediate between *Gnetum Gnemon* and Welwitschia still exist, they are to be looked for in *G. Bucholzianum* and *G. Africanum*, or in other species occurring in the same phytogeographical region, and at present undiscovered.

[It is due to the author to state that, during the last few months, he has been conducting a Sladen Botanical expedition in different parts of Africa, and has therefore had no opportunity of noticing the most recent literature bearing on the subject of this paper.—A. C. S.]

Botanical Laboratory,
South African College, Cape Town.

* Engler, 1908.
† Rendle, 1899.
‡ Bolus, 1905.
§ Pearson, 1907 (*b*).
‖ Pearson, 1907 (*b*), 1908.

APPENDIX I.

My colleague, Prof. A. BROWN, has kindly calculated the number of nuclei in the sac, after the completion of the last of the series of simultaneous mitoses, from the median longitudinal section shown in fig. 30. The method employed, which is of general application to such cases, is here given.

A median line A_1 A_{11} (see fig. 30) is drawn, and divided into 10 equal parts; ordinates are drawn at each of the points of section and measured; call them y_1 y_2 ... y_{11}. The area of the section is taken as

$$2A_1A_{11}\times\left(\frac{y_1}{2}+y_2+y_3+y_4+y_5+y_6+y_7+y_8+y_9+y_{10}+\frac{y_{11}}{2}\right)\times\frac{1}{10}=S,$$

and the volume of the solid as

$$\pi\,.\,A_1A_{11}\times\left(\frac{y_1^2}{2}+y_2^2+y_3^2+y_4^2+y_5^2+y_6^2+y_7^2\times y_8^2+y_9^2+y_{10}^2+\frac{y_{11}^2}{2}\right)\times\frac{1}{10}=V,$$

these being elementary approximations.

If n be the number of nuclei in the section, S/n is the mean surface to one nucleus. If the distribution is uniform we may assume $(S/n)^{\frac{3}{2}}$ to be the mean volume to a nucleus, hence the number of nuclei in the volume is $V/(S/n)^{\frac{3}{2}}$, *i.e.*, $\frac{Vn^{\frac{3}{2}}}{S^{\frac{3}{2}}}$.

The measurements give $A_1A_{11} = 10{\cdot}1$ cm.,

and the y's 0, 1·0, 1·25, 1·31, 1·32, 1·30, 1·16, 1·04, 0·84, 0·60, 0,

giving $S = (2\times10{\cdot}1\times0{\cdot}98)$ sq. cm.,

$V = (\pi\times10{\cdot}1\times1{\cdot}12)$ c.c.

Also $n = 185$.

number in the volume is

$$(185)^{\frac{3}{2}}\times\pi\times1{\cdot}12\times10{\cdot}1^{-\frac{1}{2}}\times0{\cdot}98^{-\frac{3}{2}}\times2^{-\frac{3}{2}},$$

log 185 = 2·2672	3·4008	0·5021
log 0·98 = 1·9912	0·4969	$\bar{1}$·9868
	0·0492	0·4515
	3·9469	0·9404
	0·9404	
	3·0065	

Giving a number, 1015.

APPENDIX II.

Methods.

As far as possible the fixing was done in the field at the time of collection. The winged perianth was in all cases removed from the more advanced ovules before they were placed in the solutions. The apex of the cone-axis with its attached bracts and very young flowers was fixed as a whole. In cases in which the fixation had to be deferred, sometimes for a period as long as three hours, the results compare more favourably than might be expected with those yielded by the material fixed when collected.

For the stages of the embryo-sac preceding fertilisation, by far the best results were obtained with a solution of picric acid and mercuric chloride in a 5-per-cent. solution (alcoholic) of glacial acetic acid.*

The use of a solution of chromic and osmic acids in 4-per-cent. glacial acetic acid† (aqueous) was hardly less successful, especially for embryo-sac-tubes, pollen-tubes, and sexual nuclei.

The bulk of the material was very satisfactorily fixed in a solution of chromic and acetic acids, according to a formula used by Mr. I. B. Pole Evans, viz. :—

Chromic acid (1-per-cent. solution in distilled water) . . .	200 c.c.
Glacial acetic acid	1 c.c.
Distilled water	50 c.c.

Several other reagents were also employed, *e.g.*, saturated solution of mercuric chloride in 1-per-cent. acetic acid; acetic alcohol (30 : 70); Carnoy's solution; mixtures of absolute alcohol and formaline and aqueous solutions of the latter. Except for the purposes of comparison the material preserved in these has not been studied. None of them appear to yield results as good as those obtained with the first three formulæ.

Great difficulty was experienced in washing material after fixation. Apparatus was prepared for immersing it in the river at Haikamchab, but as this was quite dry it could only be washed by frequent changes of water or alcohol. It was then taken up to 70 per cent. alcohol or Calberla's fluid. Later, in the laboratory, it was again washed in changes of alcohol.

The preparations were made by the paraffin-method. Most of the drawings are made from sections 8 μ thick. The hæmatoxylins (Heidenhain, Ehrlich, and Delafield) alone or in combination with alcoholic eosin or erythrosin, combinations of diamant-fuchsin and *licht-grün* and of cyanin and erythrosin were the principal

* Chamberlain, 1905, p. 20.

† Chamberlain, 1906, p. 327.

stains used. Diamant-fuchsin and *licht-grün* have been especially valuable in the investigation of the pollen-tubes and embryo-sac-tubes. The former is the only stain that has made clear the structure of the starch grains.

Difficulties, which have not yet been overcome, have attended the investigation of the nuclear changes intervening between the stages of the microspore and the pollen-grain. The wall of the microspore is already so thick that stains penetrate very slowly, and this, no doubt, also applies to the fixing reagents.* The hardness of the wall and the small size of the grain has made it impossible to obtain good sections. The only method which has yet been attended with any degree of success has been to stain in bulk for several days in Ehrlich's hæmatoxylin, and afterwards to extract the excess of colouring matter by means of acid alcohol. But the nuclear structures are always obscured by the opacity of the wall, even after clearing.

The drawings have been made under the *camera lucida*; in all cases Zeiss achromatic objectives and Huygenian eyepieces have been used.

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* PEARSON, 1906, figs. 12–16.

† I am indebted to Dr. B. DAYDON JACKSON, Sec. L.S., for a photograph of this paper.

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* Miss E. L. STEPHENS, B.A., has kindly furnished me with an abstract of this paper.

DESCRIPTION OF PLATES.

KEY.

Figs. 1– 7	Plate 22	Figs. 37–44	Plate 26
„ 8–18	„ 23	„ 45–55	„ 27
„ 19–28	„ 24	„ 56–64	„ 28
„ 29–36	„ 25	„ 65–80	„ 29

Figs. 81–89 . . . . . Plate 30.

A. *Extra-floral Embryo-sacs.*—(Fig. 1.)

Fig. 1.—Outline of median longitudinal section through the apical portion of a female cone showing the positions of the embryo-sacs figured in figs. 1A and 1B (A, B), and the summit of that shown in 1C (C). *pr.* = procambial strand. × 39.

Fig. 1, A, B, C.—The embryo-sacs indicated in fig. 1, A, B, × 520; C, × 305. *ax.* = sterile tissue of cone-axis; *e. s.* = embryo-sac.

B. *Microspore-mother-cell and Microspores.*—(Figs. 2–7.)

Fig. 2, × 305; Fig. 7, × 1250; the rest, × 1700.

Fig. 2.—Part of a section through a microsporangium whose mother-cell-nuclei are in the prophase of the heterotypic division. *ep.* = epidermis; *m. w., i, w.* = outer and inner tapetum; *mi. m. c.* = microspore-mother-cell.

Fig. 3.—Prophase of heterotypic division.

Fig. 4.—Telophase of heterotypic division.

Fig. 5.—Equatorial-plate-stage of homotype divisions.

Fig. 6.—Anaphase.

Fig. 7.—Cell-plate formation.*

C. *Structures concerned in Pollination.*—(Figs. 8–11.)

Figs. 9, 10, × 305; fig. 11, × 86.

e. s. t. = embryo-sac-tube; $ep._1$ = outer layer of nucellar cone; $ep._2$ = inner layer of integument; *int.* = integument; *n.* = nucellus; *p. g.* = pollen-grains; *p. t.* = pollen-tube.

Fig. 8A.—Photograph of male cones *in situ* showing projecting stamens (from PEARSON, 1907 (*a*)).

Fig. 8B.—Photograph of female cones *in situ* showing projecting micropyles, some of which are tipped with drops of the micropylar fluid.

* For later stages of microspore and pollen-grain see PEARSON, 1906, figs. 9–16.

Fig. 9.—Part of longitudinal section of young (unpollinated) ovule passing through integument and edge of nucellar cone.

Fig. 10.—Part of transverse section of pollinated ovule passing through integument and outer part of nucellar cone.

Fig. 11.—Longitudinal section through lower part of micropylar tube and top of nucellar cone, showing numerous pollen-grains and pollen-tubes.

D. *Macrospore-mother-cell, Macrospore, and Embryo-sac.*—(Figs. 12–23.)

m. w. = macrospore-wall; *n.* = nucellus.

Fig. 12.—Macrospore-mother-cell with nucleus in prophase of heterotypic division (probably recovery from synapsis). × 1700. (*Cf.* Pearson, 1906, fig. 20.)

Fig. 13.—A macrospore in longitudinal section. × 700.

Fig. 14, A, B.—Macrospore-nucleus in mitosis (two successive sections), showing about 25 chromosomes. *v.* = polar vacuoles. × 700.

Fig. 15.—Bi-nucleate embryo-sac showing a tendency towards a polar arrangement of the nuclei. × 700.

Fig. 16.—Embryo-sac with four nuclei arranged as a tetrad. × 700.

Fig. 17.—Embryo-sac from an old cone showing a great development of vacuoles and most of the nuclei arranged peripherally. × 700.

Fig. 18.—Embryo-sac showing 12 free nuclei in simultaneous mitosis. × 700.

Fig. 19.—Three nuclei from a sac (older than that of the last figure), in which all the nuclei are in simultaneous mitosis. × 1700.

Fig. 20.—A later stage. × 305. (*Cf.* Pearson, 1906, fig. 30.)

Fig. 21.—An embryo-sac containing the full number of free nuclei. × 305. (*Cf.* Pearson, 1906, fig. 31.)

Fig. 22.—A median longitudinal section through the micropylar end of the sac showing the planes of septation. All these compartments will form embryo-sac-tubes into which their nuclei will pass. × 700.

Fig. 23.—Part of longitudinal section through the lower three-fourths of the same sac as fig. 22. The planes of septation are similar to those shown in fig. 22. The compartments are smaller and contain more nuclei. If none of these contain so few as six nuclei they will all form uninucleate cells of the primary endosperm. × 700.

E. *The Micropylar End of the Sac after Septation and the Embryo-sac-tubes.*—(Figs. 24–31.)

Fig. 27, × 180; the rest, × 700.

end. = endosperm; *e. s. t.* = embryo-sac-tube; *m. w.* = macrospore-wall; *n* = nucellus; *st.* = starch grains.

Fig. 24.—Part of longitudinal section showing last stages of wall-formation and free sexual nuclei. (*Cf.* PEARSON, 1906, fig. 32A.)

Fig. 25.—A later stage in which the outgrowth of embryo-sac-tubes has commenced. One compartment contains five nuclei. (*Cf.* PEARSON, 1906, fig. 33A.)

Fig. 26.—A median longitudinal section through the tissue of the nucellar cone lying immediately above the embryo-sac and into which young embryo-sac-tubes have penetrated. One tube contains five free sexual nuclei which lie close together near the tip.

Fig. 27.—A somewhat tangential longitudinal section through the upper part of the embryo-sac and the superincumbent nucellar tissue showing embryo-sac-tubes, one of which is growing downwards.

Fig. 28.—An older embryo-sac-tube containing two sexual nuclei lying some distance behind the tip.

Fig. 29.—A similar tube containing four nuclei, a little contracted.

Fig. 30, A and B.—Very exceptional cases in which the nuclei in embryo-sac-tubes have fused. The fusion-nuclei closely resemble that of an endosperm-cell. (*Cf.* fig. 35.)

Fig. 31.—A more advanced tube in which the nuclei are separated.

F. *The Lower Three-fourths of the Sac after Septation and the Primary Endosperm.*—(Figs. 32–39.)

Figs. 38B and 39, × 305; the rest, × 700.

f = cell containing a fusion-nucleus; other lettering as in previous section.

Fig. 32.—Same sac as fig. 24. Multinucleate compartments distinctly marked out, but cell-walls not yet visible. (*Cf.* PEARSON, 1906, fig. 32B.)

Fig. 33.—Same sac as fig. 25. Cell walls distinct. In some compartments the nuclei have already fused (*f*). A single nucleus in mitosis (*a*). (*Cf.* PEARSON, 1906, fig. 33B.)

Fig. 34.—In one multinucleate compartment the eight nuclei are in simultaneous division (five seen in the section); one contains a fusion-nucleus; the two small cells have been formed by the division of a fusion-nucleus.

Fig. 35, A–D.—Stages in the fusion of the nuclei to form uninucleate endosperm-cells; in C, a primary endosperm-nucleus is in mitosis.

Fig. 36.—A longitudinal section through a part of the primary endosperm in which nuclear fusion is completed. In one cell is seen a stage in the formation of a cell-plate.

Fig. 37, A and B.—A, an embryo-sac-tube containing a seven-celled tissue (*end.*) identified as an endosperm formed from a fusion-nucleus such as those shown in fig. 30. B, a group of small cells from the normal endosperm of the same ovule drawn to the same scale.

Fig. 38, A and B.—A, a portion of a longitudinal section through the antipodal end of the same sac as fig. 26, showing a compartment with free nuclei surrounded by uninucleate endosperm-cells. B, A deep-seated compartment in which the two nuclei have not fused (only one appears in the section); its position is shown at α in fig. 89H.

Fig. 39.—A longitudinal section passing through the junction between two embryo sacs in the same ovule situated one above the other. No nuclear fusion has yet occurred in the upper one (*a*); in the lower, all the compartments contain fusion-nuclei except a few (*e. s. t.*), situated at the side where the pressure is least (see p. 354).

G. *Germination of the Pollen-grain and Growth of the Pollen-tube.*—(Figs. 40–52.)

Fig. 41, × 1250; fig. 47, × 22; the rest, × 700.

end. = endosperm; *ex.* = exine; *g. c.* = generative cell; *ch.* = chalazal tissue formed below the integument by intercalary growth; *in.* = intine; *int.* = integument; *n.* = nucellus; *per.* = "perianth"; *p. t.* = pollen-tube; *t. n.* = tube-nucleus.

Fig. 40.—Pollen-grain in early stage of germination.

Fig. 41.—Part of pollen-tube showing elongated generative cell. The tube bends sharply at "*a*" to enter the summit of the nucellus.

Fig. 42.—A cell of the nucellar cone showing compound starch grains.

Fig. 43, A and B.—Pollen-tube shortly after entering the nucellus, showing generative cell and tube-nucleus.

Figs. 44, 45.—Two successive sections through a pollen-tube showing the nucleus of the generative cell in mitosis. The tube-nucleus is seen in fig. 45.

Fig. 46.—A pollen-tube after the mitosis of the nucleus of the generative cell.

Fig. 47.—Outline of longitudinal section of an ovule showing the course of a pollen-tube into the chalazal region (*f*).

Fig. 48.—The generative cell whose position (*g. c.*) is indicated in fig. 47.

Fig. 49.—A generative cell. A deeply stained mass seen in the next section, lying just in front of the generative cell, represents the tube-nucleus. The leading male nucleus is smaller than the one behind it (as is usually the case) and here stains more deeply.

Fig. 50, A and B.—Two successive sections of a pollen-tube showing two approximately equal male nuclei and a tube-nucleus in process of absorption.

Fig. 51.—A generative cell. The second nucleus is greatly elongated.

Fig. 52.—A generative cell. The leading nucleus is much smaller than the hinder one, stains very deeply, shows little internal structure and has an irregular outline. It is regarded as being in process of disintegration.

H. *Meeting of the Embryo-sac-tube and Pollen-tube, and Fertilisation.*—(Figs. 53–67.)

Figs. 53, 60A, × 305 ; fig. 64, × 1250 ; the rest, × 700.

a. f. = aborting female nucleus ; other lettering as in preceding section.

Fig. 53.—Part of a longitudinal section through the nucellus showing an embryo-sac-tube and a pollen-tube approaching one another.

Fig. 54.—Parts of the tubes of fig. 62 enlarged. The female nucleus shows an anterior papilla. A mass of cytoplasm containing a degenerating nucleus, five sections away from this one, is probably the second half of the generative cell.

Fig. 55.—Embryo-sac-tube and pollen-tube meeting (the tip of the pollen-tube destroyed in preparation). The female nucleus shows an anterior papilla, and is ensheathed in a thin layer of deeply staining cytoplasm. The second half of the generative cell, containing a male nucleus in normal condition, is present in the succeeding section.

Fig. 56.—A bi-nucleate generative cell and, in front of it, a nucleus which is identified as ♀. The lower part of the tube (*e. s. t.* ?) is continuous below with a canal in which lies a two-celled pro-embryo. This is interpreted as a case in which one of the lower female nuclei has been fertilised before the leading one. This is unusual.

Fig. 57.—An embryo-sac-tube and pollen-tube in contact. The female nucleus has entered the cytoplasm of the pollen-tube. The male nuclei in the generative cell are in normal condition. A second female nucleus can be identified in this embryo-sac-tube below the limit of the figure.

Fig. 58.—Three of four successive sections of the same embryo-sac- and pollen-tubes (one section between B and C is not shown). In A, the leading female nucleus is within the generative cell ; the second female nucleus within the cytoplasm of the pollen-tube and a tangential section of the generative cell are seen in B ; C shows a third female nucleus still in the embryo-sac-tube, and some distance below that of B. The second male nucleus has not been identified.

Fig. 59.—A large generative cell containing an elongated nucleus ; the second male nucleus has disappeared, unless it is represented by the deeply stained line in the middle of the generative cell. In front is a female nucleus perhaps still within the embryo-sac-tube.

Fig. 60A.—A pollen-tube with two parts of the generative cell: the nucleus of the hinder part shows signs of disintegration. The leading half of the generative cell containing male and female nuclei is shown on a larger scale in fig. 69B.

Fig. 61.—Male and female nuclei almost in contact within the generative cell. The male nucleus shows an anterior depression. The position of the aborted male nucleus is probably shown by a small mass of deeply stained particles in the hinder part of the generative cell.

Figs. 62, 63.—Two successive sections through a nucellus, showing embryo-sac-tube and pollen-tube in communication. In fig. 63, male and female nuclei within the generative cell; in fig. 62, a second female nucleus in the embryo-sac-tube. A third female nucleus, probably belonging to the same tube, is seen in another section.

Fig. 64.—A generative cell containing a male and female nucleus in the act of fusing.

Fig. 65.—Male and female nuclei in process of fusion. The nucleoli are distinct. Part of the nuclear substance stains more deeply than the rest. The oospore-cytoplasm shows a similar differentiation; its outline is irregular and there is yet no trace of a cell-wall. An abortive female nucleus (*a. f.*) is partially embedded in the cytoplasm of the oospore.

Fig. 66.—A more advanced oospore with cell-wall. Nucleoli not yet fused.

Fig. 67.—An oospore. The cell-wall is not yet completely formed. An aborting female nucleus is seen at *a. f.*

I. *The Pro-embryo.*—(Figs. 68–87.)

Figs. 68–72, fig. 75, × 700; fig. 86 = diagrams; the rest, × 305.

c. = cap; *e.* = presumed embryonic plate; *i. c.* = terminal group of initial cells; *i. c. r.* = inner cortical ring; *i. c. t.* = inner cortical tier; *o. c. r.* = outer cortical ring; *p. s.* = primary suspensor; other lettering as in preceding section.

Fig. 68.—Young pro-embryo with nucleus in mitosis.

Fig. 69.—A larger pro-embryo, with nucleus in a later stage of mitosis. No cell-plate is visible.

Fig. 70.—The nuclear division is completed. The transverse wall is apparently formed centripetally.

Fig. 71.—A two-celled pro-embryo.

Fig. 72.—A two-celled pro-embryo, with nucleus of terminal cell in mitosis.

Fig. 73.—A three-celled pro-embryo.

Fig. 74.—A pro-embryo with a terminal group of four initial cells.

Fig. 75.—An initial cell of the stage of fig. 74 in mitosis.

Fig. 76.—A nine-celled pro-embryo with four initial cells, on the top of which lies a plate of four cells, which on division (fig. 77) give rise to the inner cortical tier of eight cells (*i. c. t.*).

Fig. 78, A and B.—The four initial cells of fig. 77 have divided longitudinally to form a group of eight, of which four are seen in A and two in B.

Fig. 79.—A later stage, showing two initial cells, and above them two of a ring of eight cells resulting from the division of the initial cells in progress in fig. 78. These divide radially to give the 16 cells of the outer cortical ring (*o. c. r.*).

Fig. 80.—The cells of the inner cortical tier have commenced to elongate. Two cells of the embryonic plate are established (E), and one of the three initial cells is in division, as a result of which a third embryonic cell will be formed. The section being oblique, three of the inner cortical ring cells are seen (also in other figures).

Fig. 81.—Two cells of the inner cortical tier, considerably elongated, are seen in longitudinal section; the inner ends of two others are cut transversely. Two cells of the outer cortical ring (*o. c. r.*) are shown and, below them, two cells of the lower ring (*x*) which have been separated by oblique walls from the terminal initial cells; the section passes through three of the cap-cells.

Fig. 82.—A later stage. The cells of the inner cortical ring (four shown) are now much elongated; those of the outer cortical ring (two) have commenced to grow. The embryonic plate, lower ring and cap, are each represented by two cells.

Fig. 83.—A tangential section of a stage a little more advanced than that of fig. 82, passing through the cap, the lower ring, the outer cortical ring, and three cells of the inner cortical ring. (It is possible that at least two of the inner cells of the tier marked "*x*" may belong to the embryonic plate, and not to the lower ring.) (*Cf.* fig. 86B.)

Fig. 84.—An oblique section through a stage hardly more advanced than that of fig. 83. Five cells of the embryonic plate (*cf.* fig. 86B) and four of the cap are shown.

Fig. 85.—A somewhat oblique section of the most advanced pro-embryo found, surrounded by endosperm tissue. The cells of the outer cortical ring have elongated considerably since the stage of fig. 84.

Fig. 86.—Diagrams of transverse sections passing through the pro-embryo of fig. 85, at the levels A, B, C.

Fig. 87. Transverse section through the secondary suspensor at the level of D in fig. 85.

K. *The Endosperm after Fertilisation.*—(Figs. 88, 89.)

Fig. 88.—Part of a longitudinal section through an endosperm after the entrance of a pro-embryo. Nuclei containing more than one nucleolus are probably primary fusion-nuclei which have not yet divided. × 180.

Fig. 89.—A series of outlines showing changes in size and form of embryo-sac and endosperm at successively older stages. A–I, × 39; K, × 22. A transverse line indicates the junction of fertile and sterile regions. Embryo-sac-tubes not shown.

sh. (figs. I, K) = shoulders of endosperm.
n. (fig. F) = inner limit of nucellus.

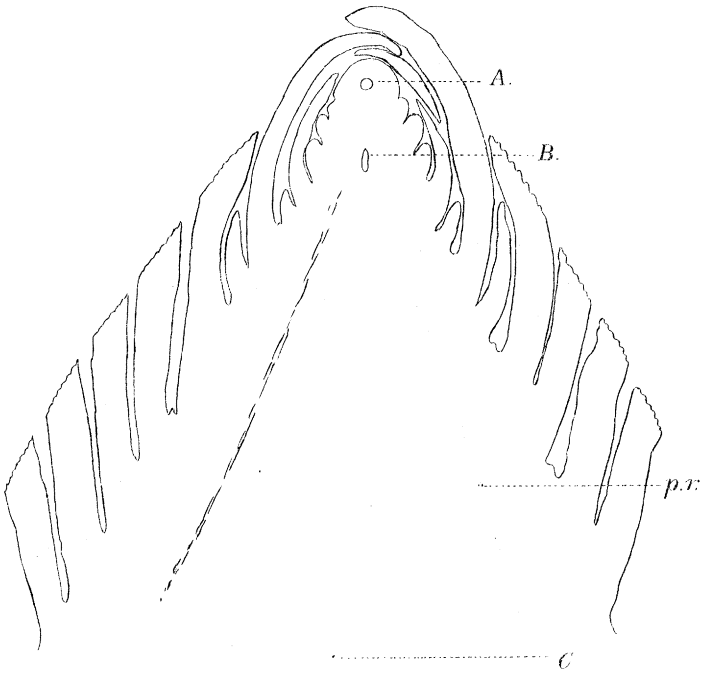
A = sac of fig. 21.
B = ,, figs. 22 and 23.
C = ,, ,, 24 and 32.
D = sac of figs. 25 and 33.
E = ,, fig. 35, A, B, D.
F = ,, ,, 36.

G = a more advanced sac yet unpollinated.

H = sac of figs. 38B and 40; pollinated but yet unfertilised. The position of the deep-seated sexual compartment of 38B is shown at α; β and γ are other sexual compartments immersed in the endosperm.

I = a later stage, after fertilisation. A pro-embryo has not yet reached the endosperm.

K = a very advanced stage containing several embryos. *c* = cavity formed by the latter (see text-figure, p. 365).



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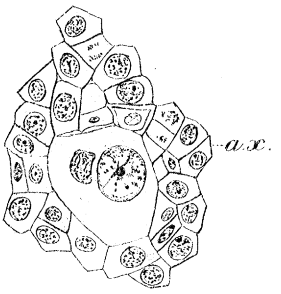

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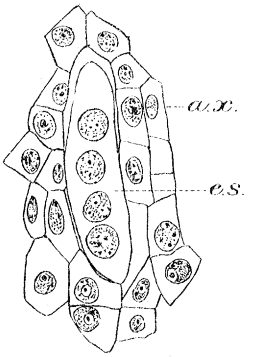

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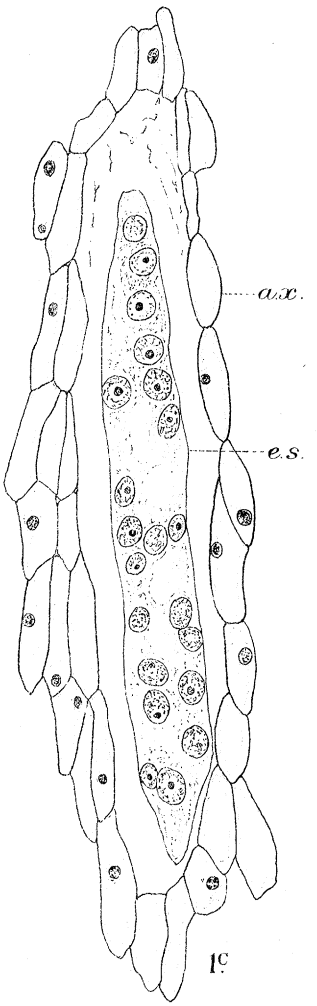

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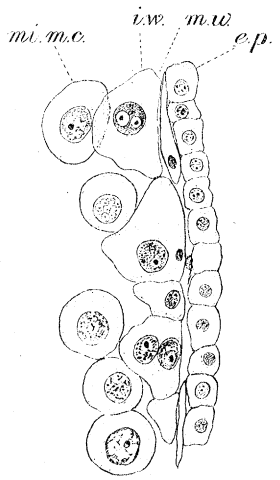

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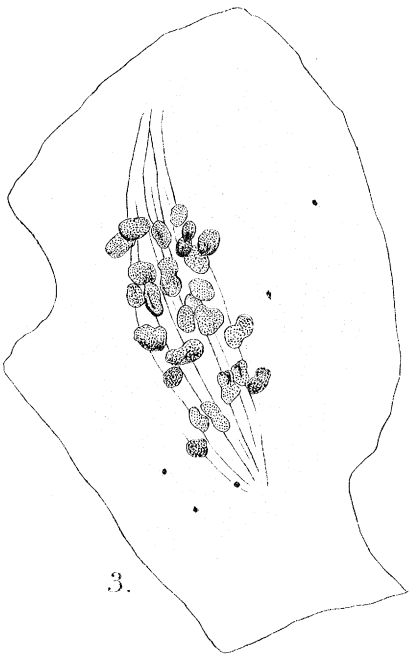

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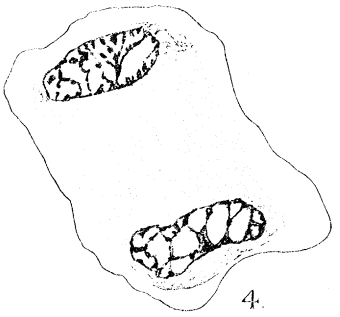

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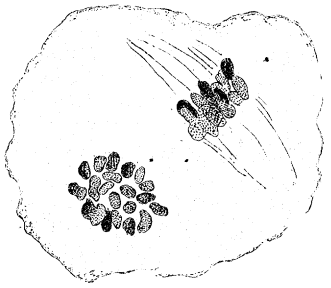

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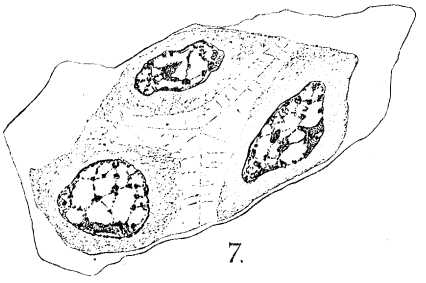

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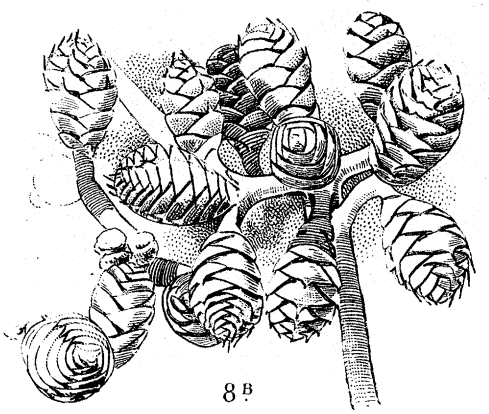

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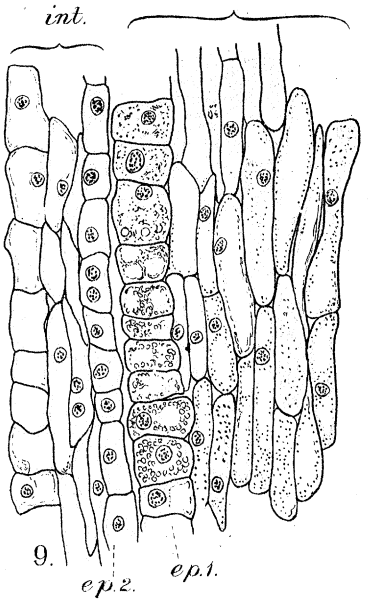

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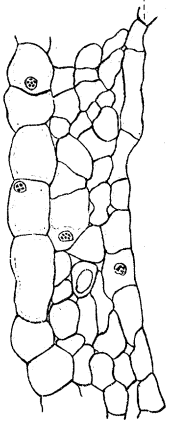

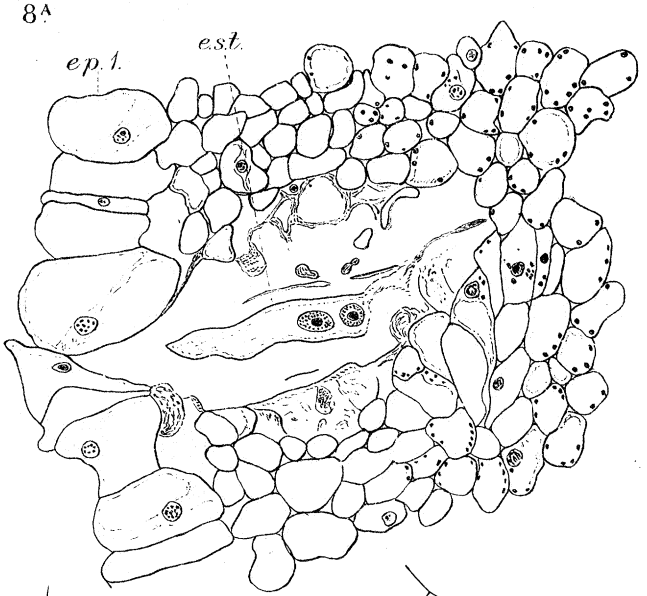

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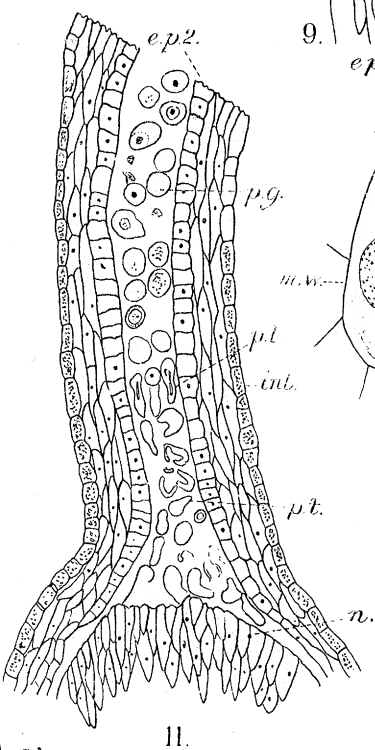

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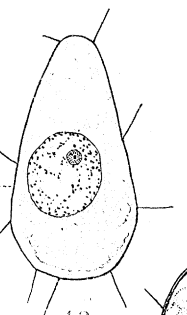

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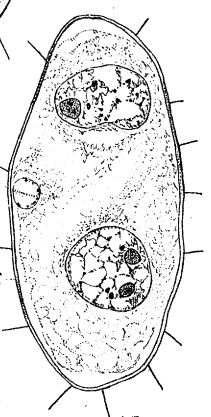

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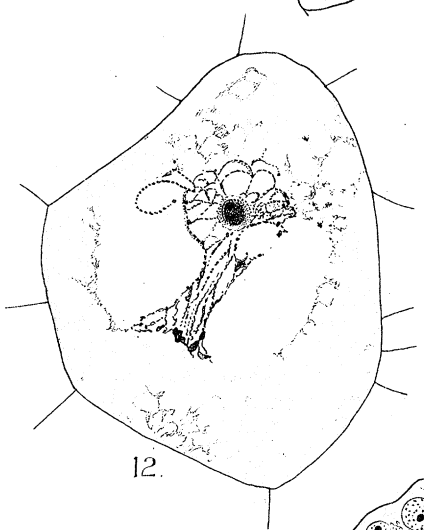

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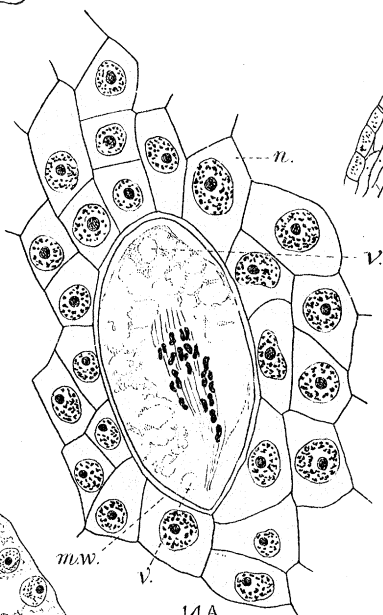

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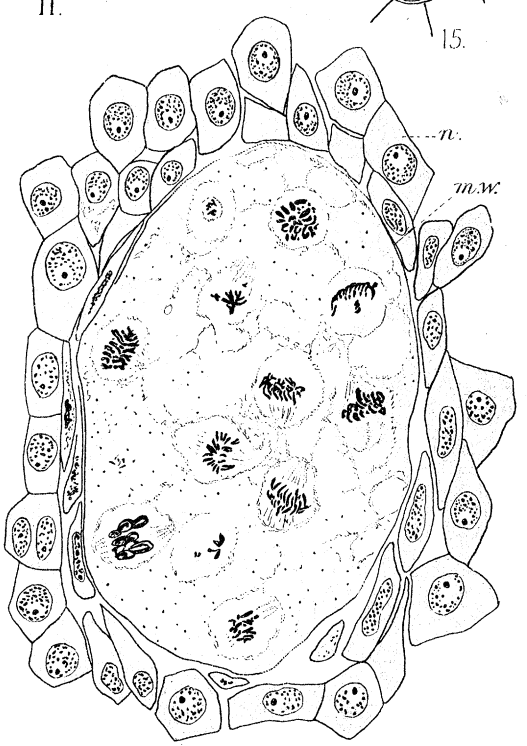

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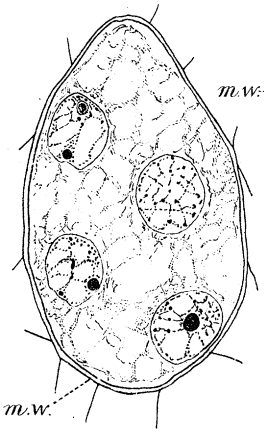

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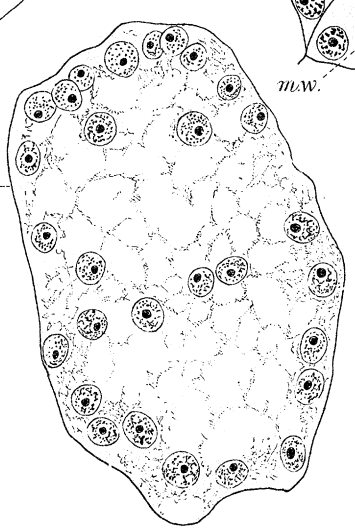

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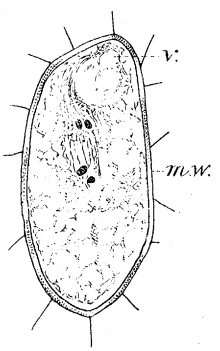

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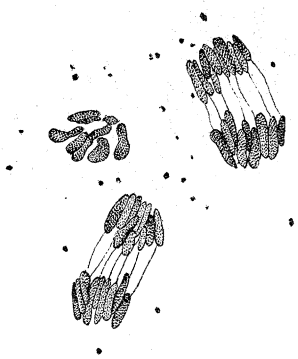

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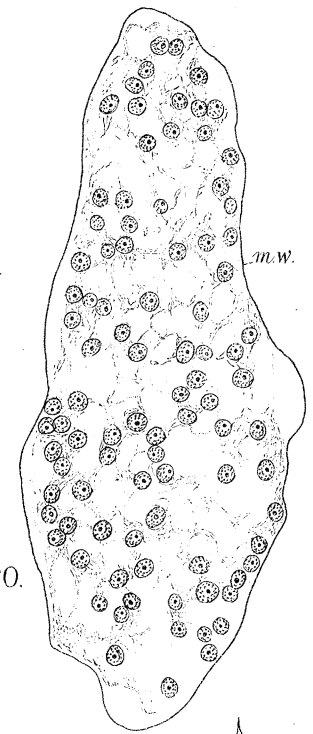

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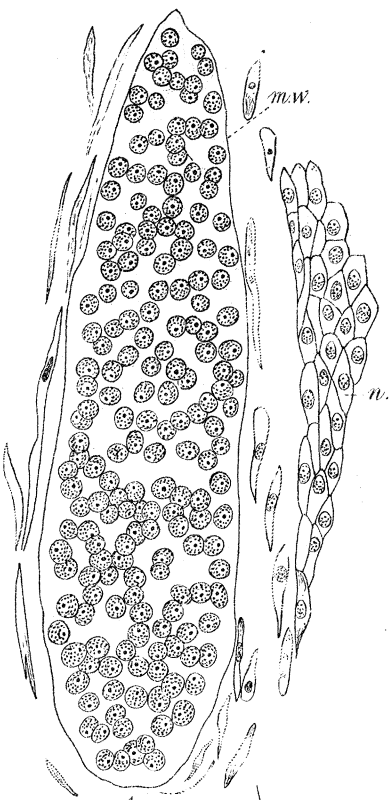

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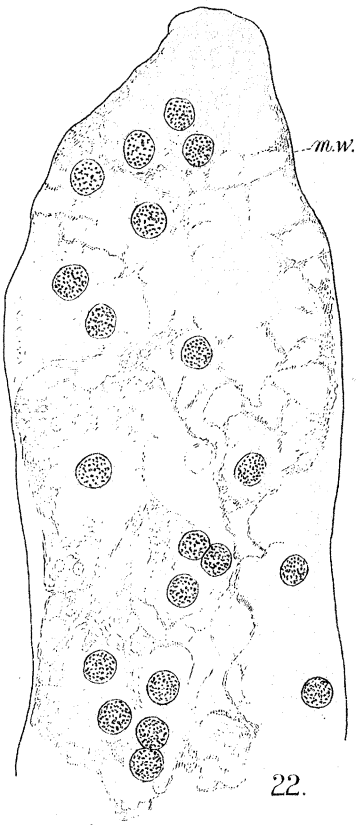

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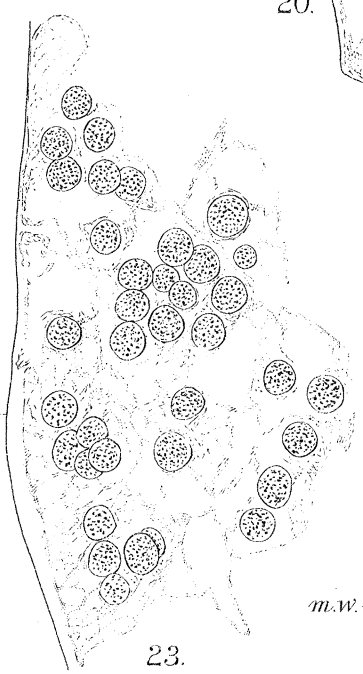

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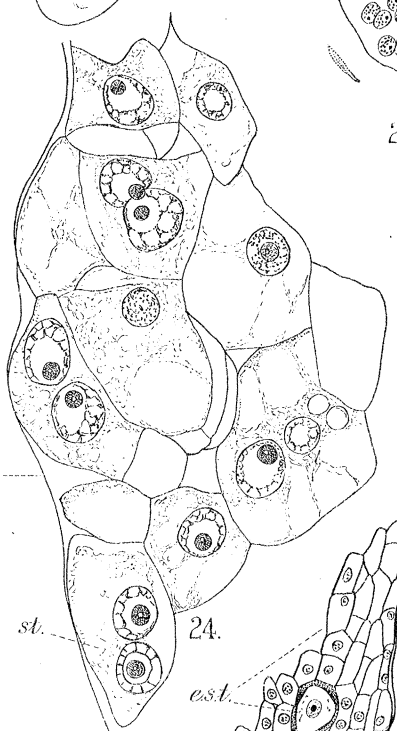

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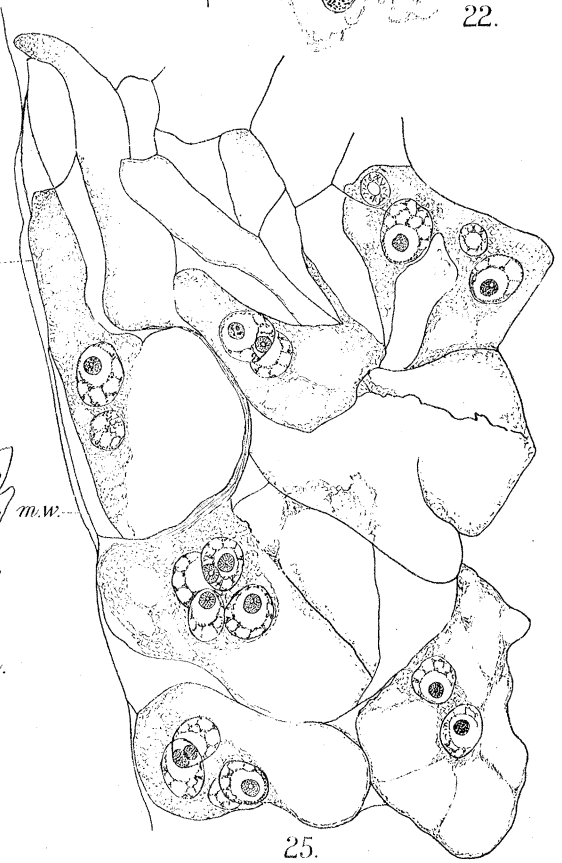

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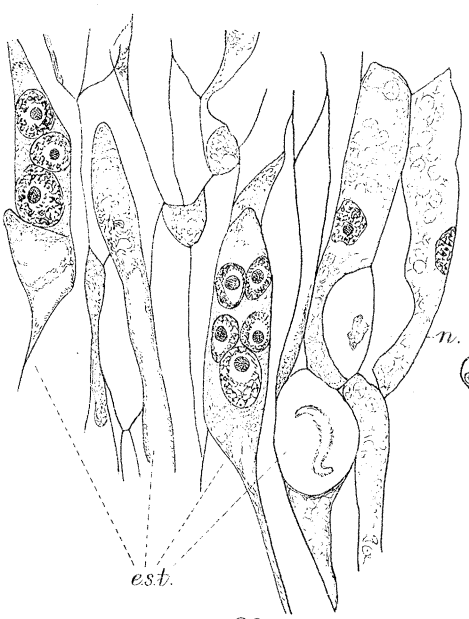

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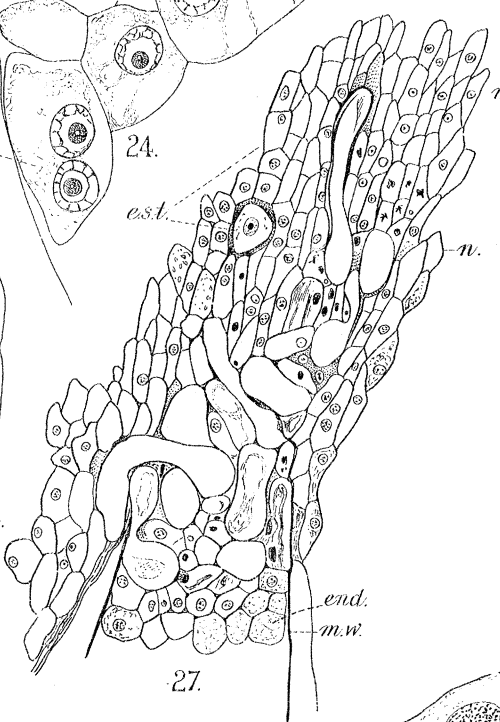

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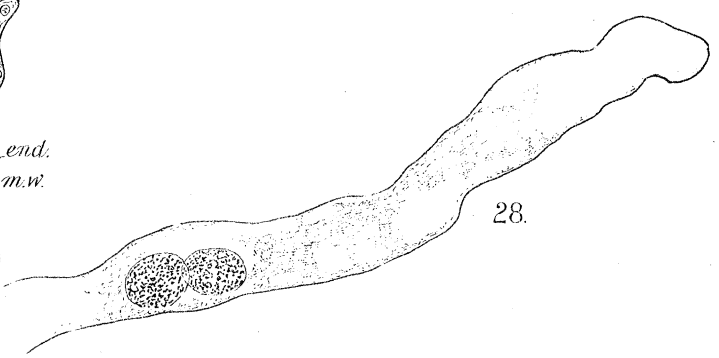

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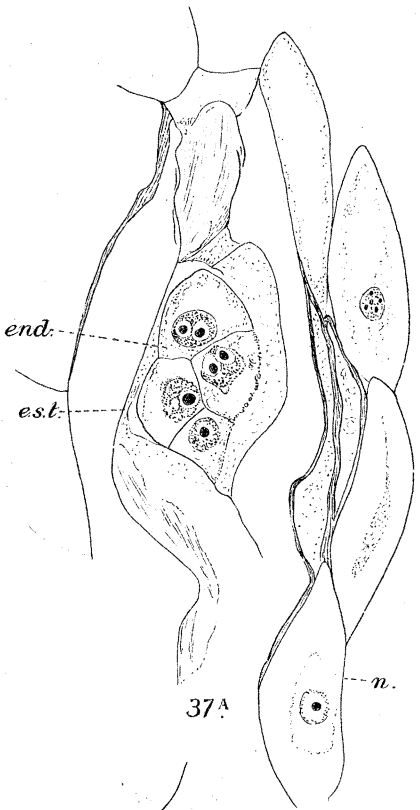

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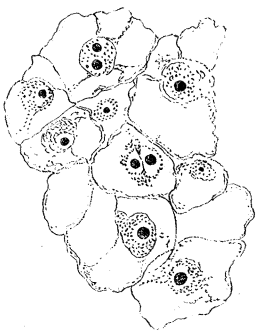

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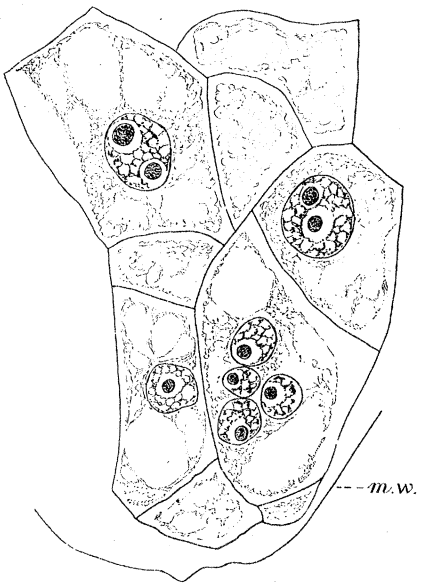

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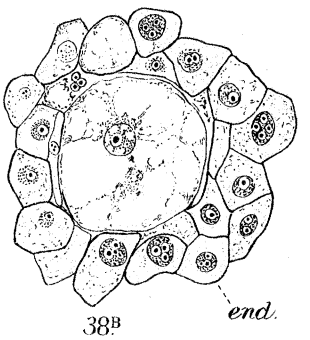

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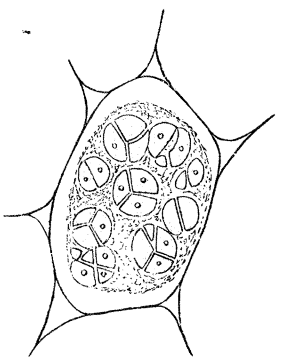

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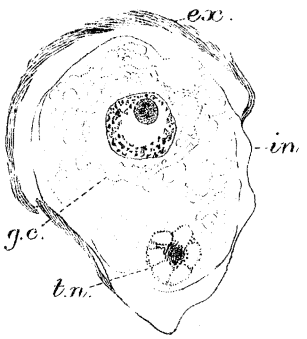

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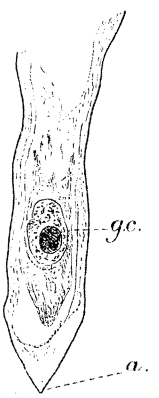

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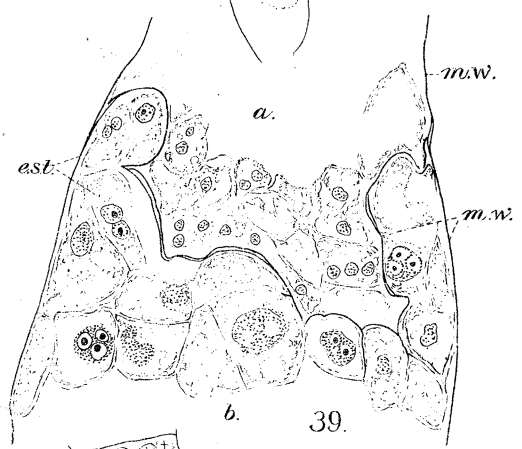

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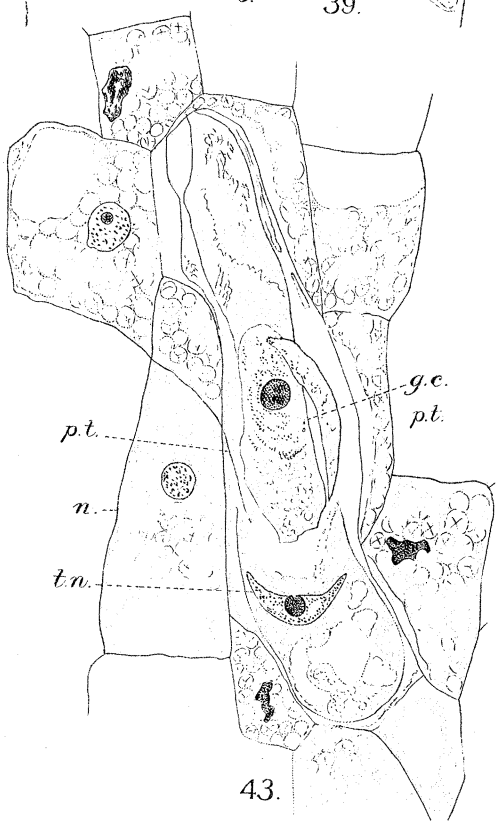

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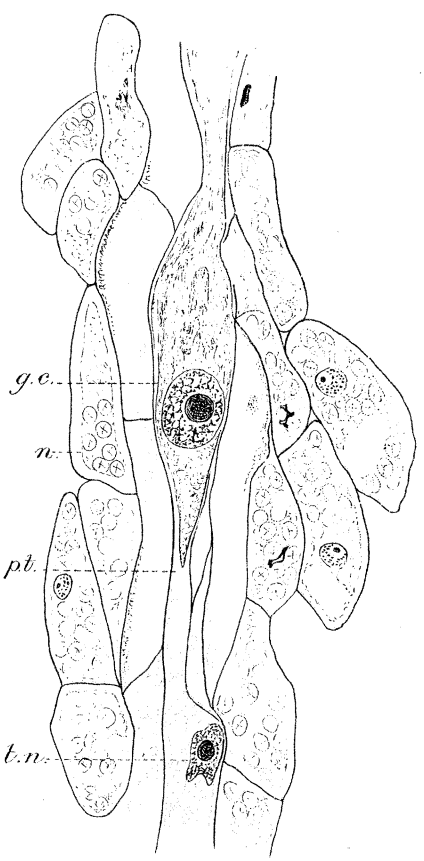

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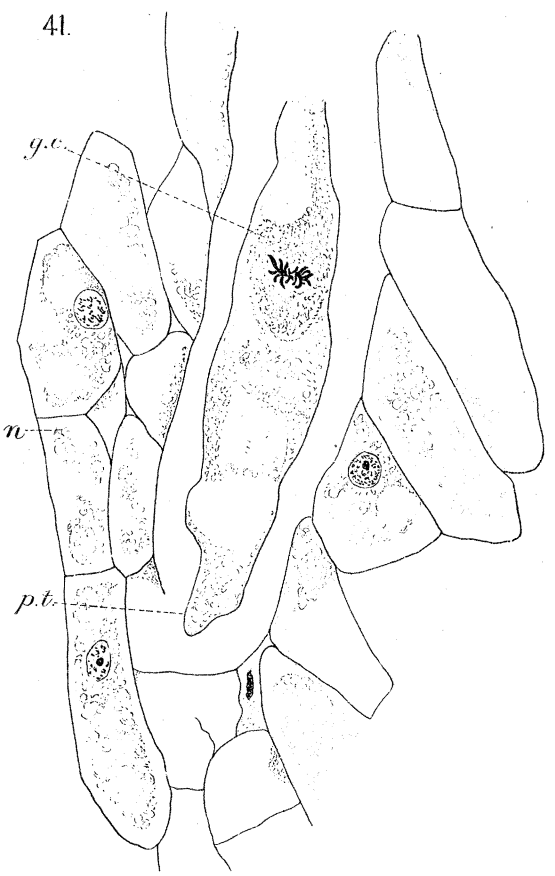

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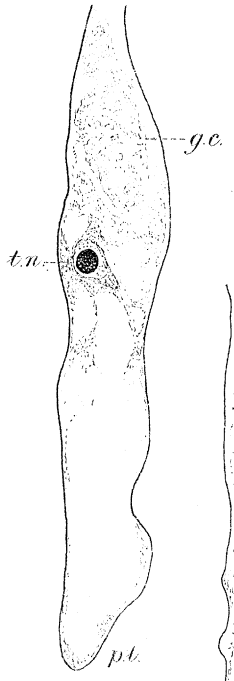

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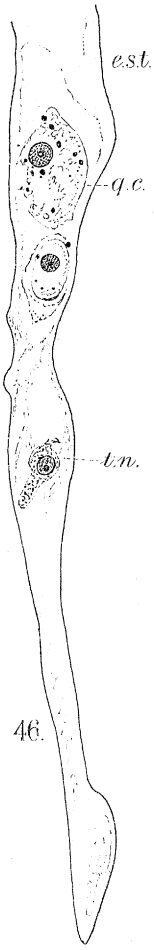

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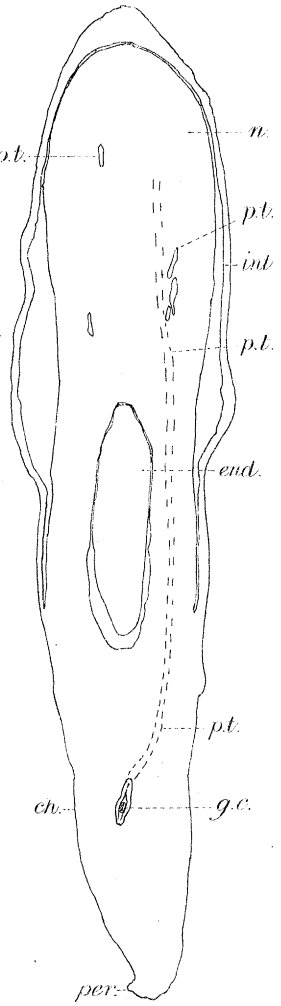

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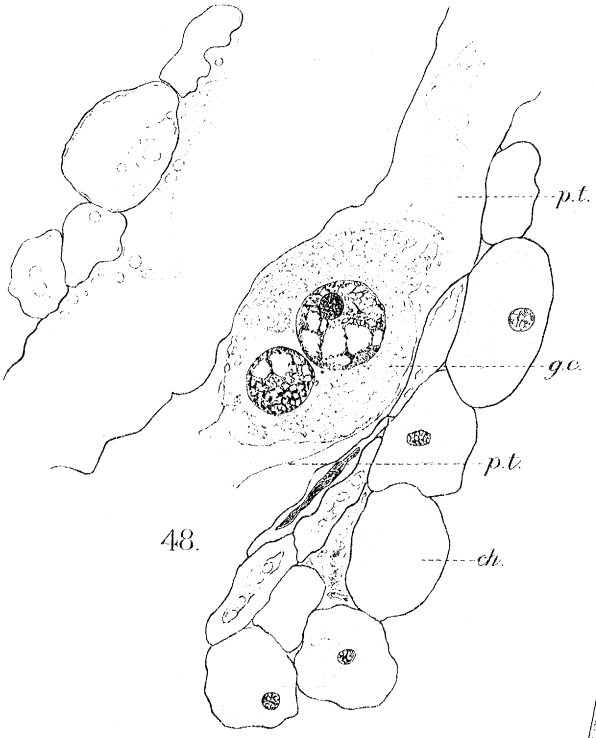

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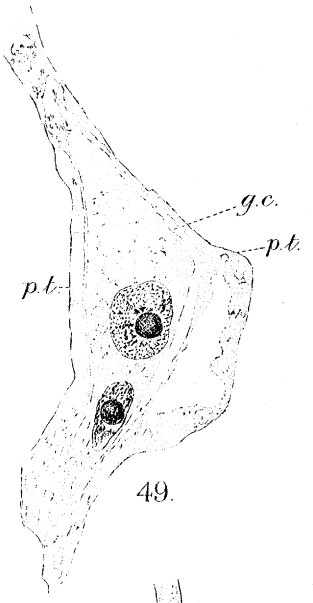

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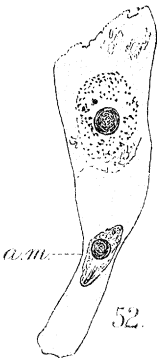

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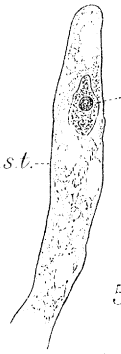

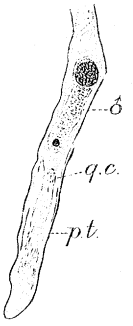

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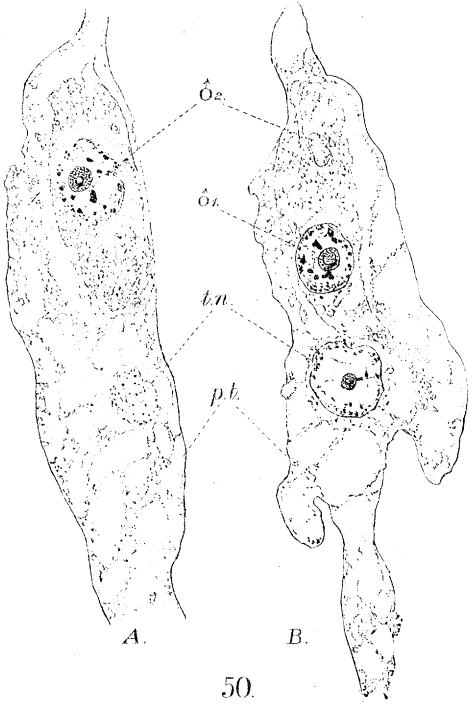

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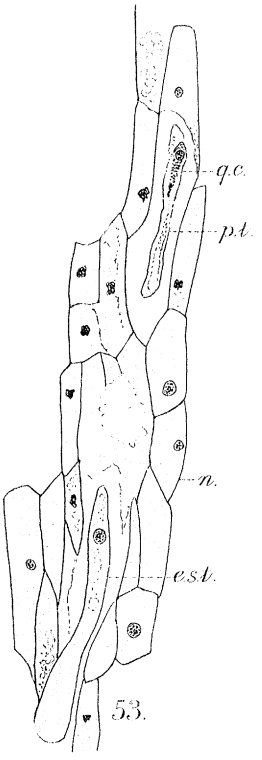

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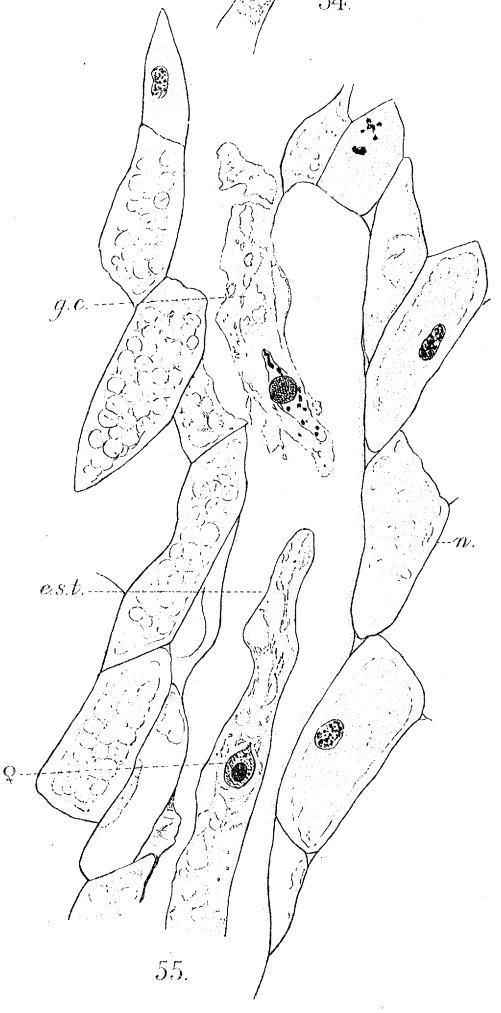

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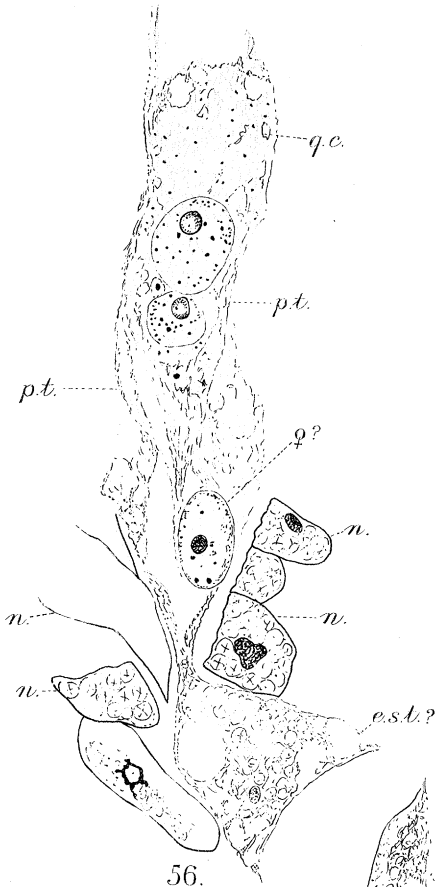

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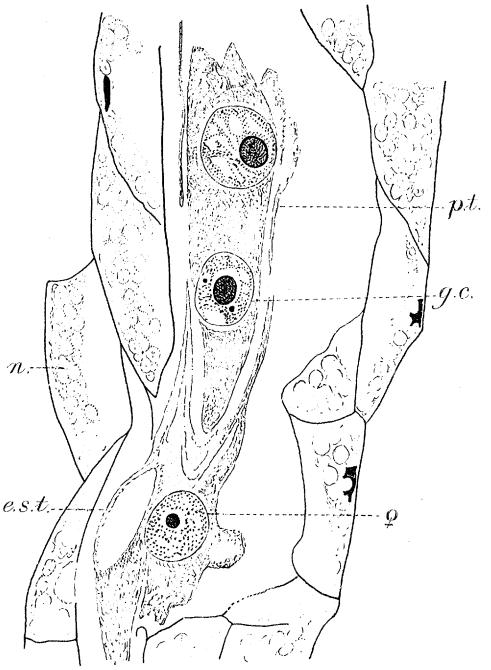

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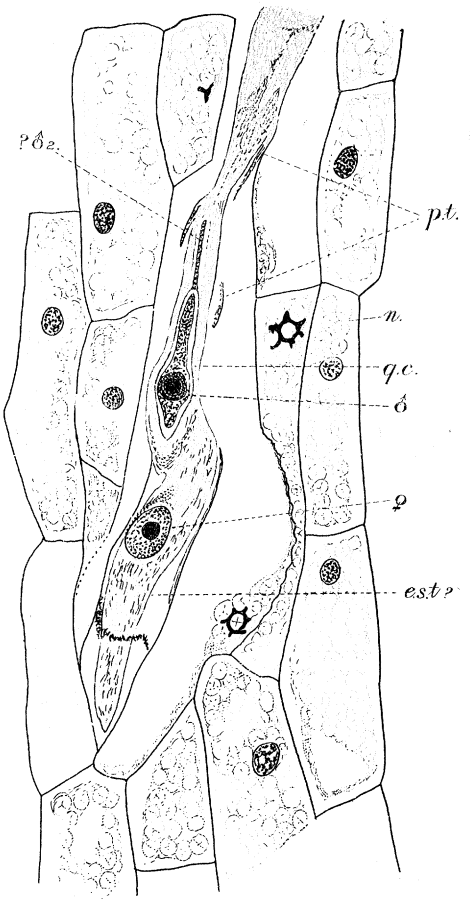

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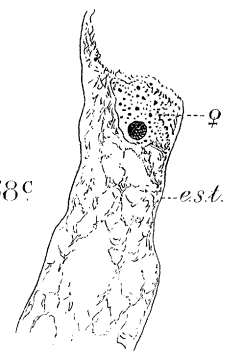

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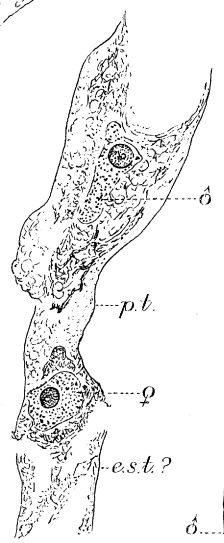

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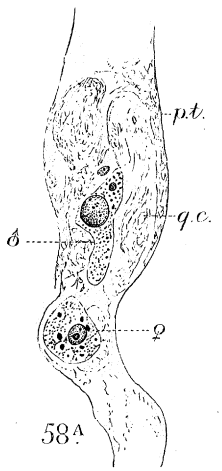

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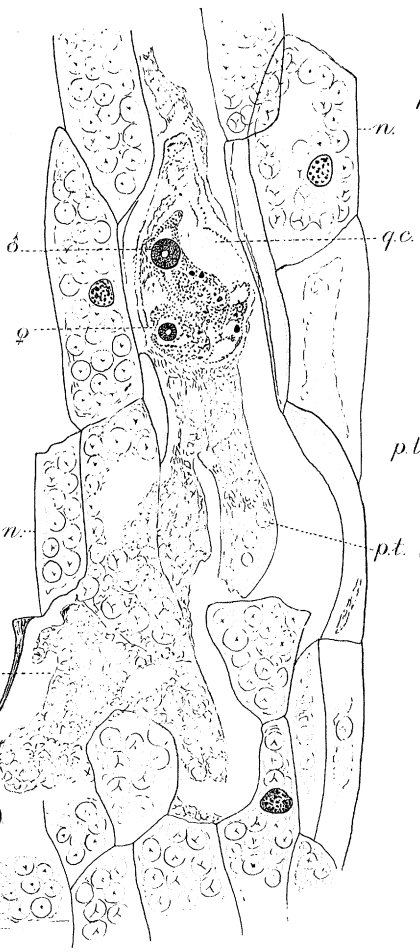

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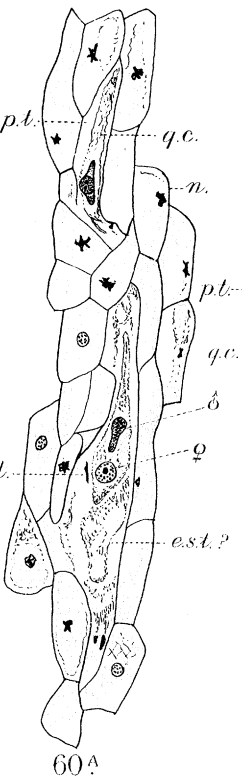

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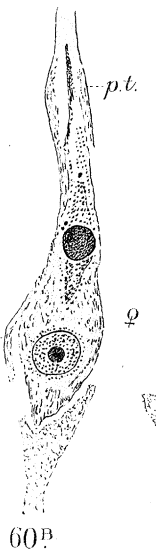

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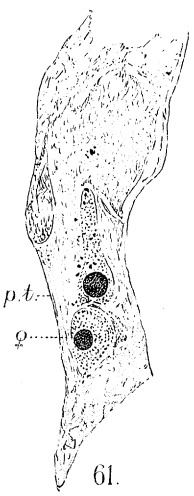

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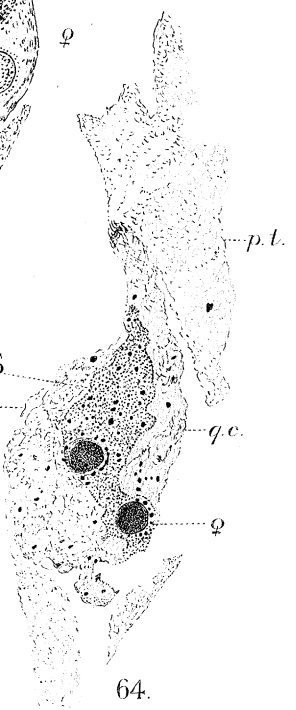

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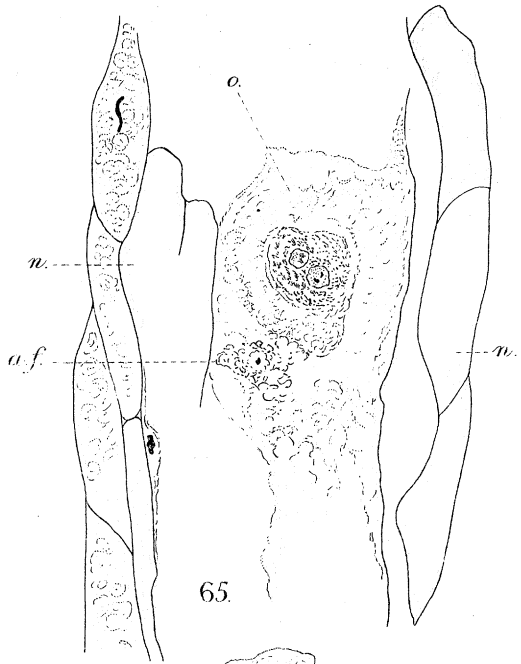

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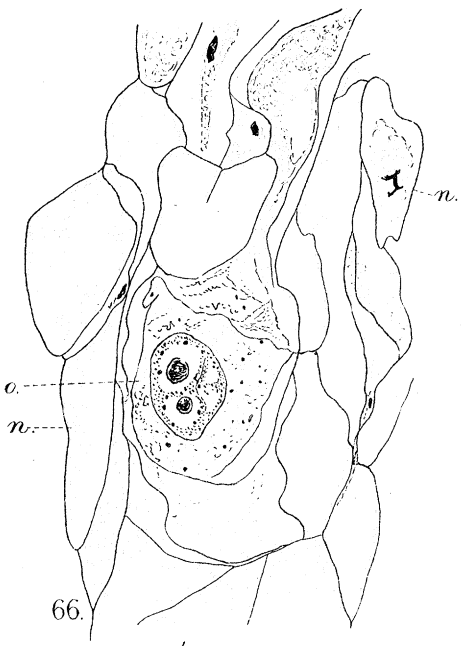

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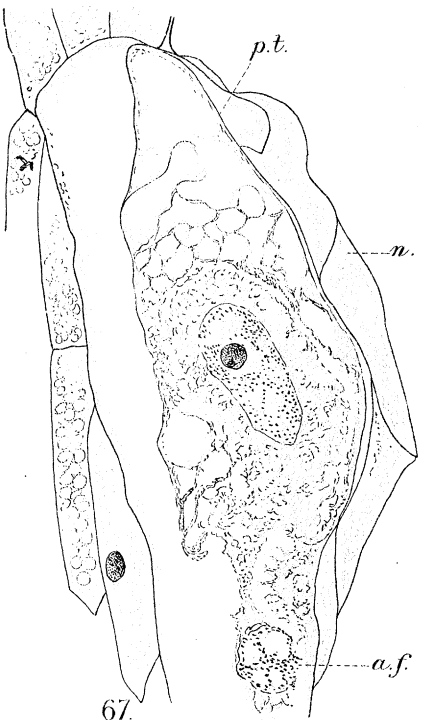

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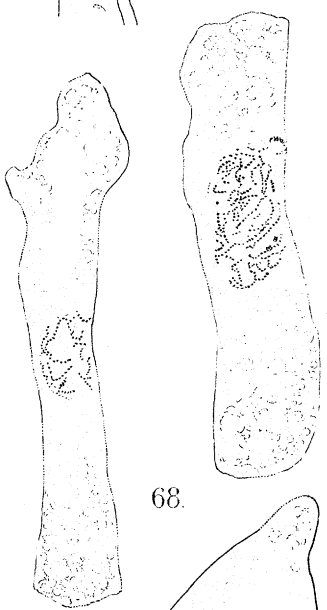

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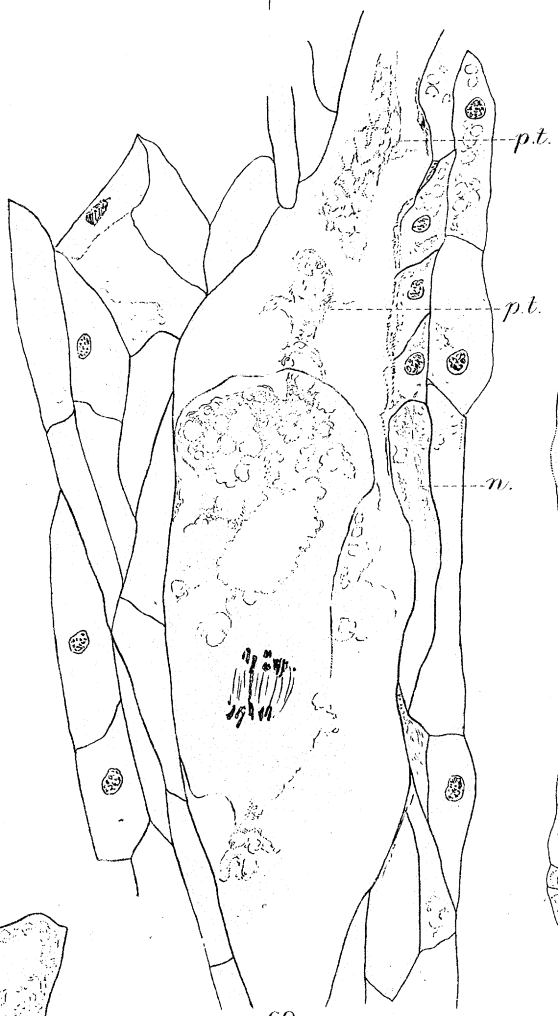

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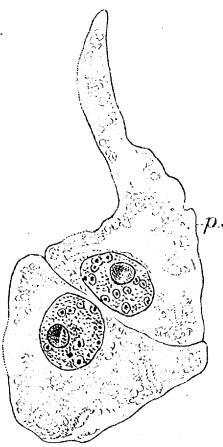

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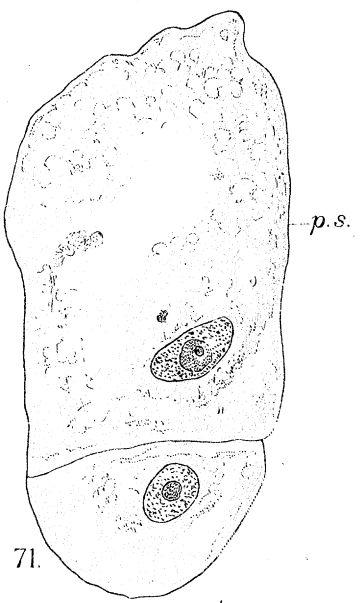

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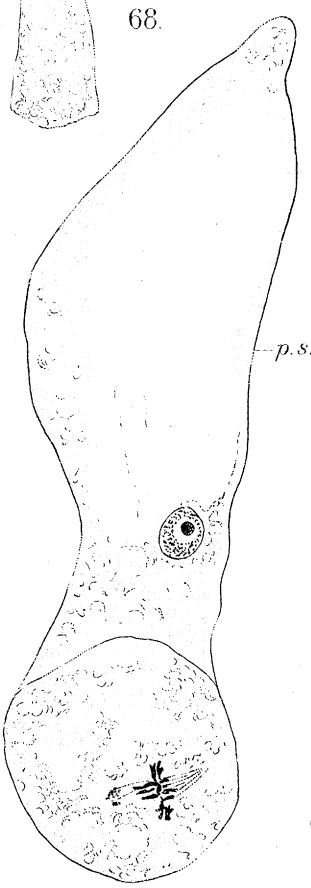

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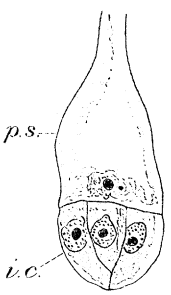

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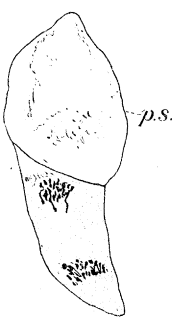

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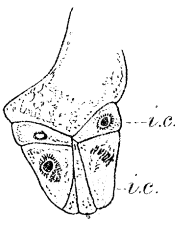

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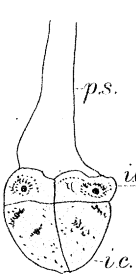

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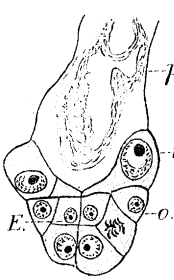

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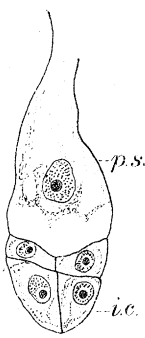

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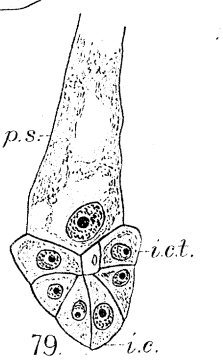

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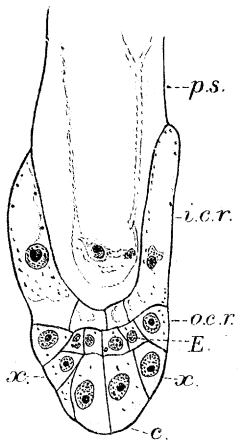

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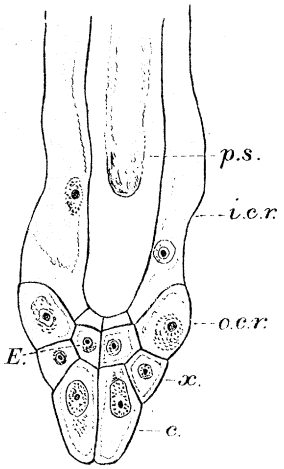

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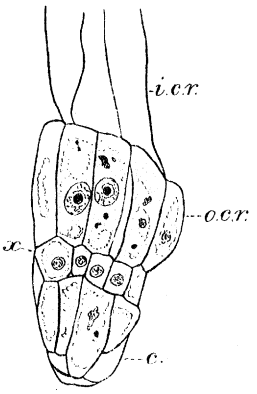

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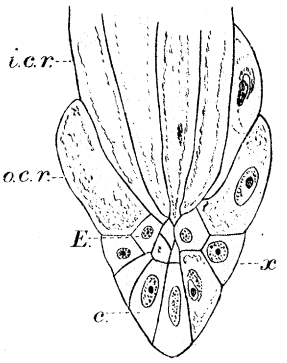

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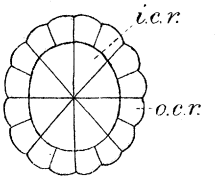

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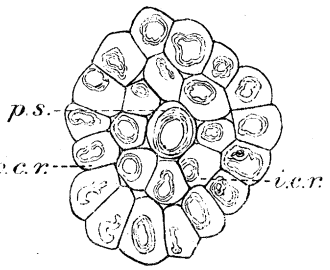

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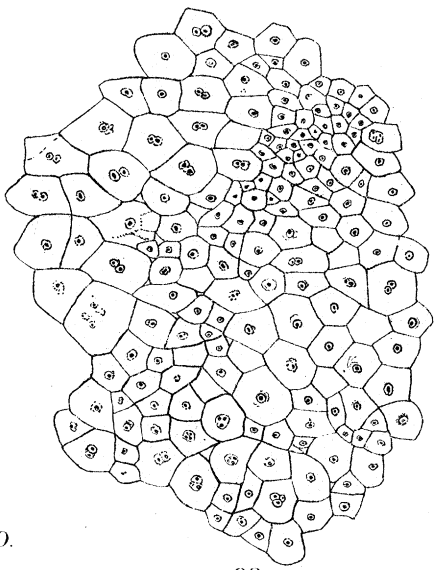

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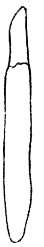

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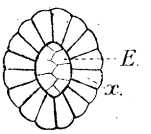

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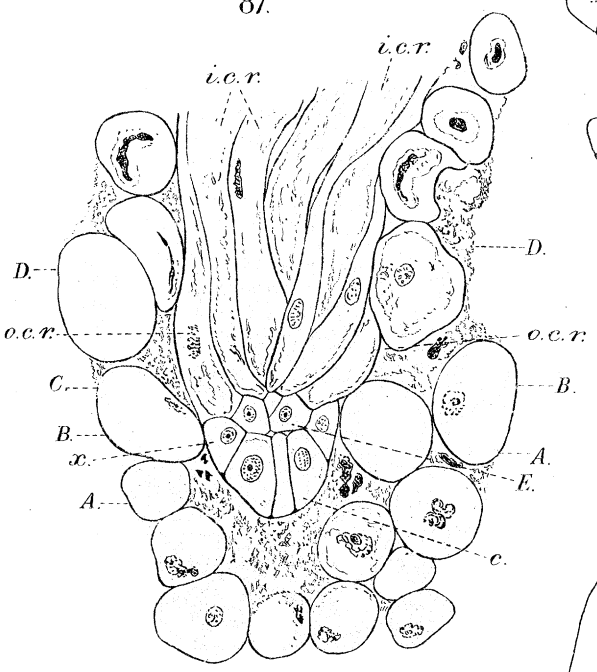

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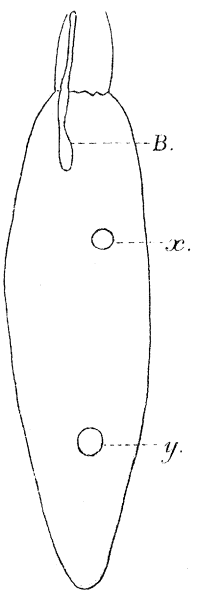

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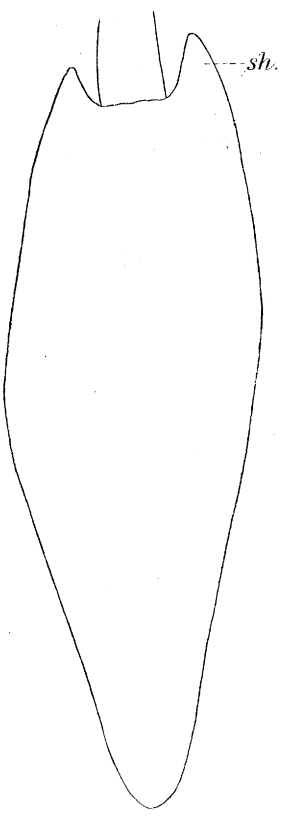

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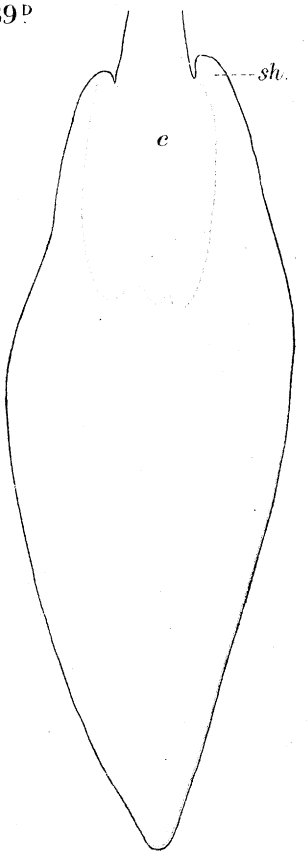

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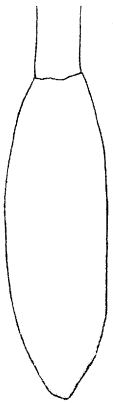

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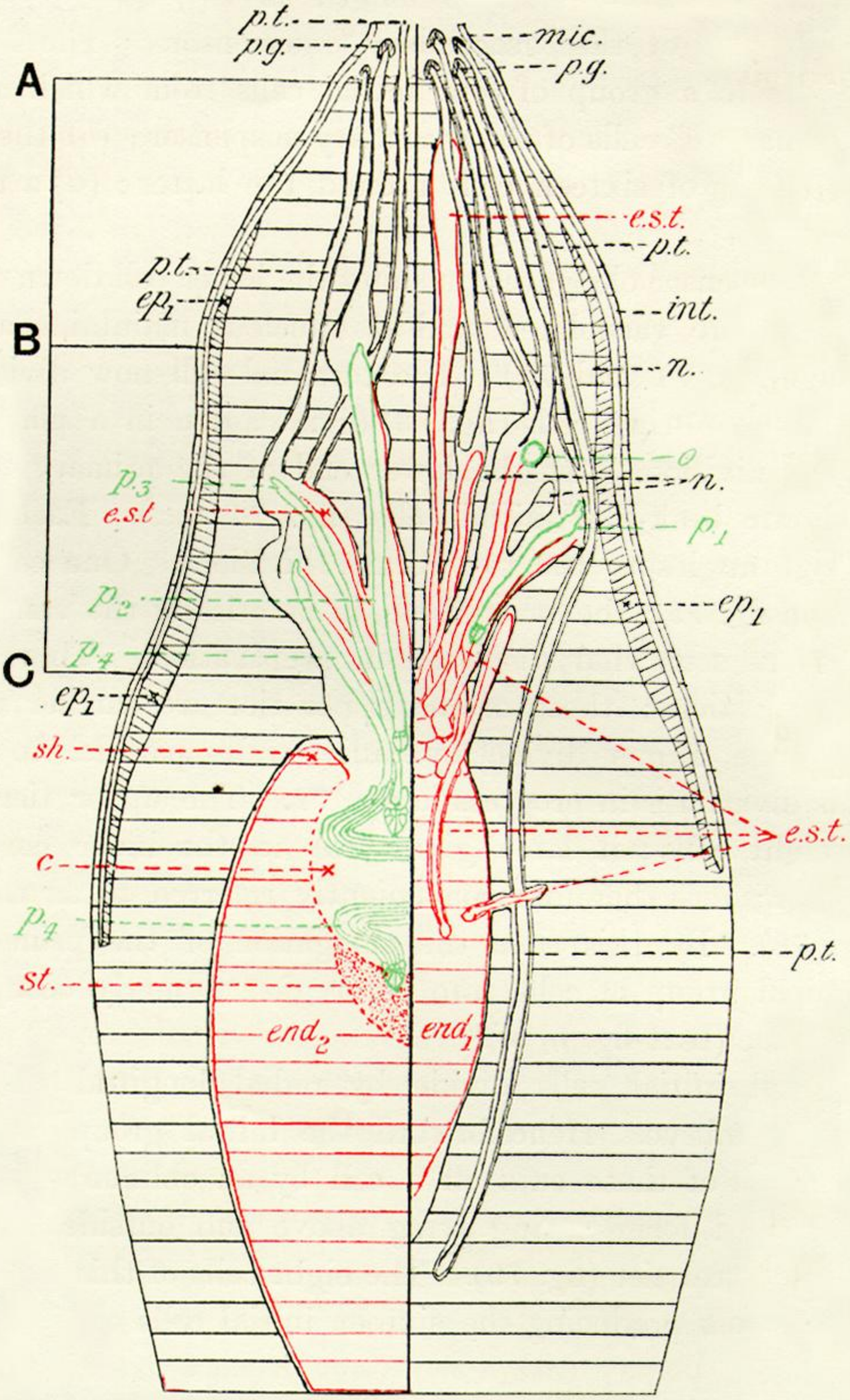
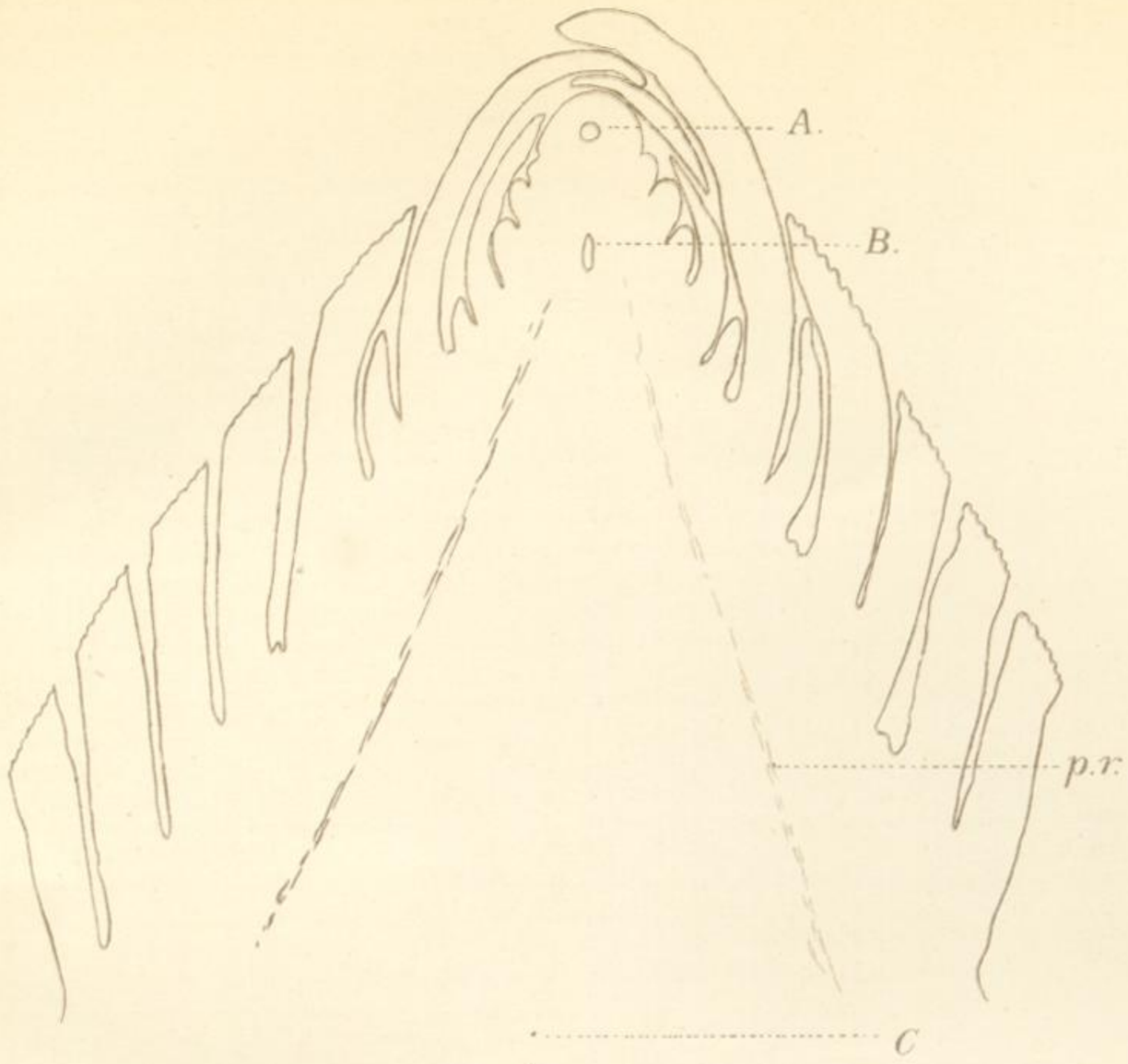


Text-figure.—A diagrammatic longitudinal section, showing halves of two ov ıles, one at the stage of fertilisation (on the right), the other containing advanced pro-embryos (on the left). The breadth is more highly magnified than the length.

c. = cavity in endosperm caused by the entrance of several pro-embryos; *end.*$_1$, *end.*$_2$ = primary and secondary endosperm; *ep.*$_1$ = outer (epithelial) layer of nucellar cone (*cf.* figs. 9, 10, *ep.*$_1$); *e.s.t.* = embryo-sac-tube; *int.* = integument; *mic.* = micropyle; *n.* = nucellus; *o.* = oospore; *p.*$_1$ = 3-celled pro-embryo (*cf.* fig. 73); *p.*$_2$ = 9-celled pro-embryo (*cf.* fig. 76); *p.*$_3$ = later pro-embryo (*cf.* fig. 81); *p.*$_4$ = advanced pro-embryo (*cf.* fig. 85); *p.g.* = pollen-grain; *p.t.* = pollen-tube; *sh.* = shoulder of secondary endosperm; *st.* = region of the secondary endosperm in which the cells contain more starch than is present elsewhere.



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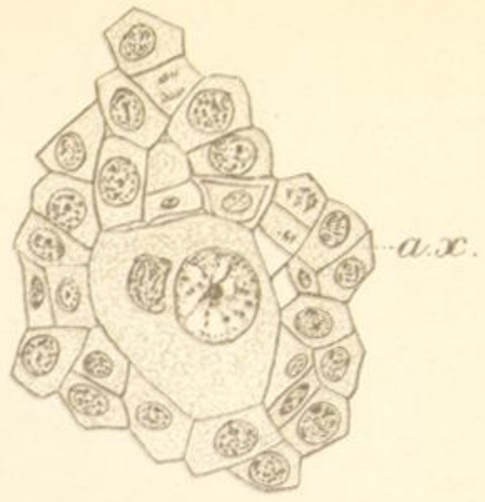

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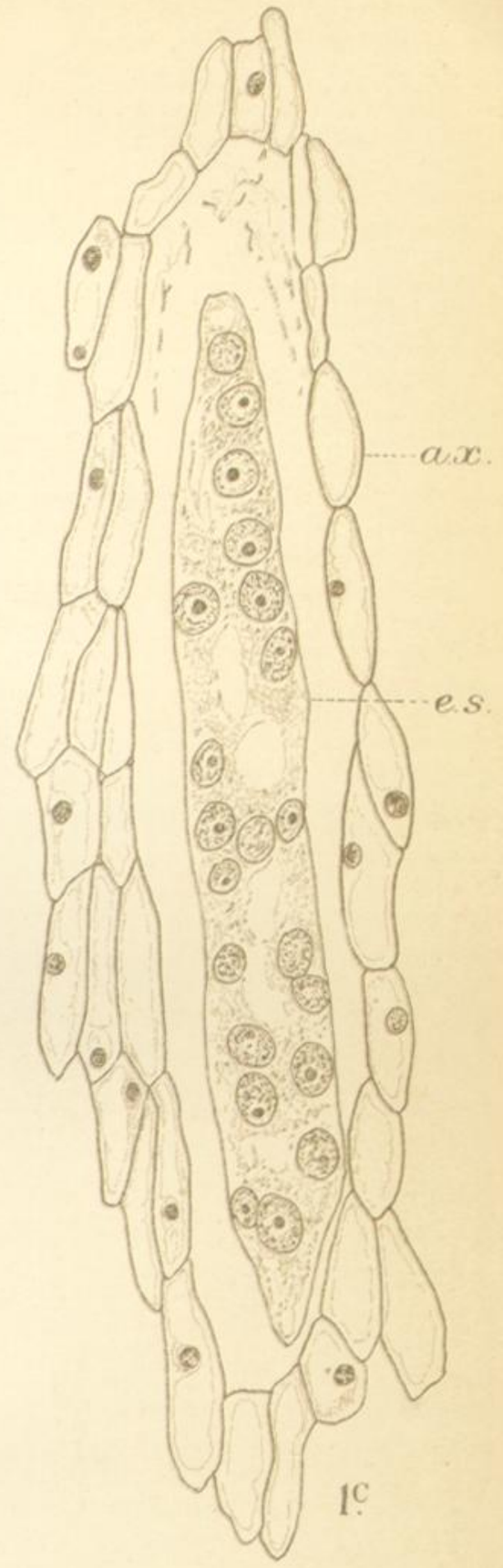

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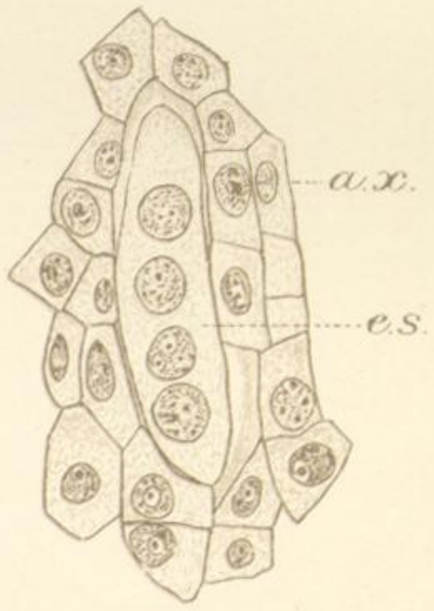

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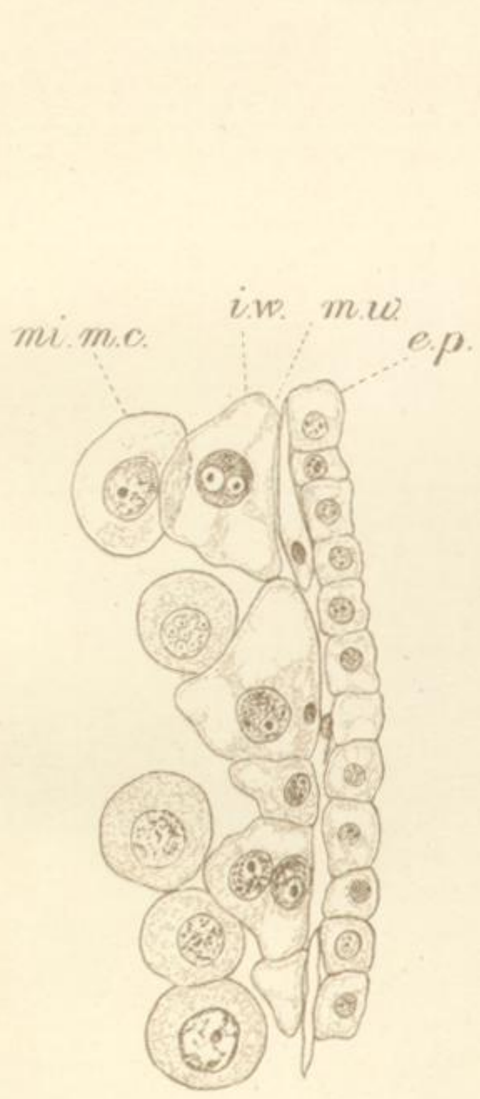

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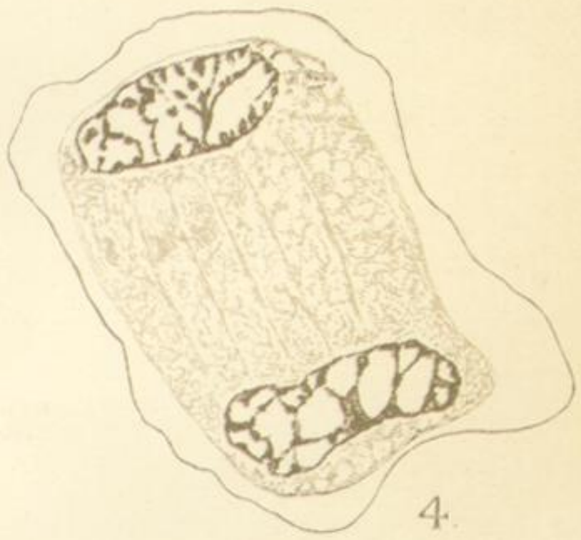

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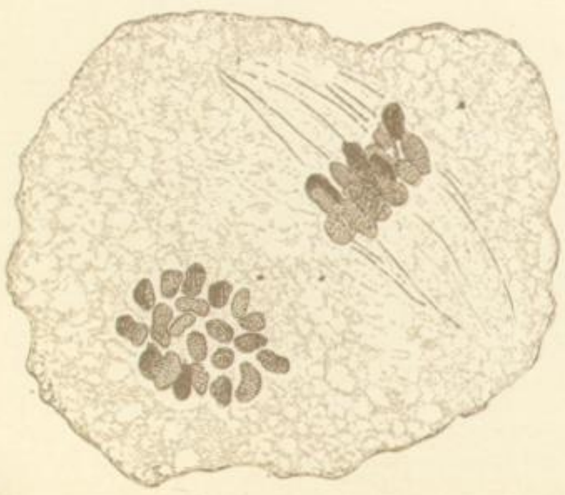

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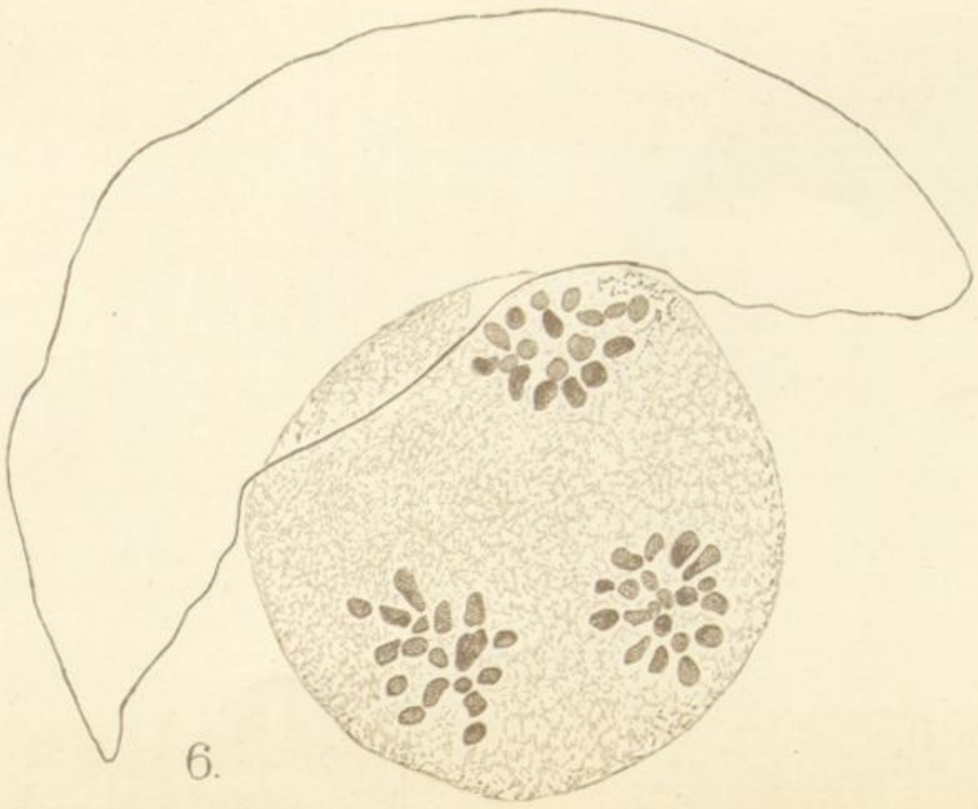

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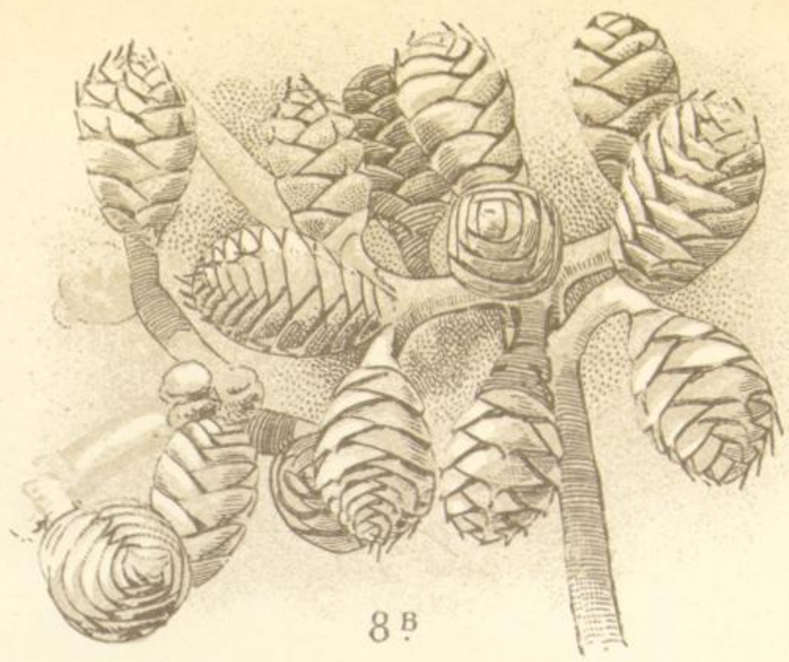

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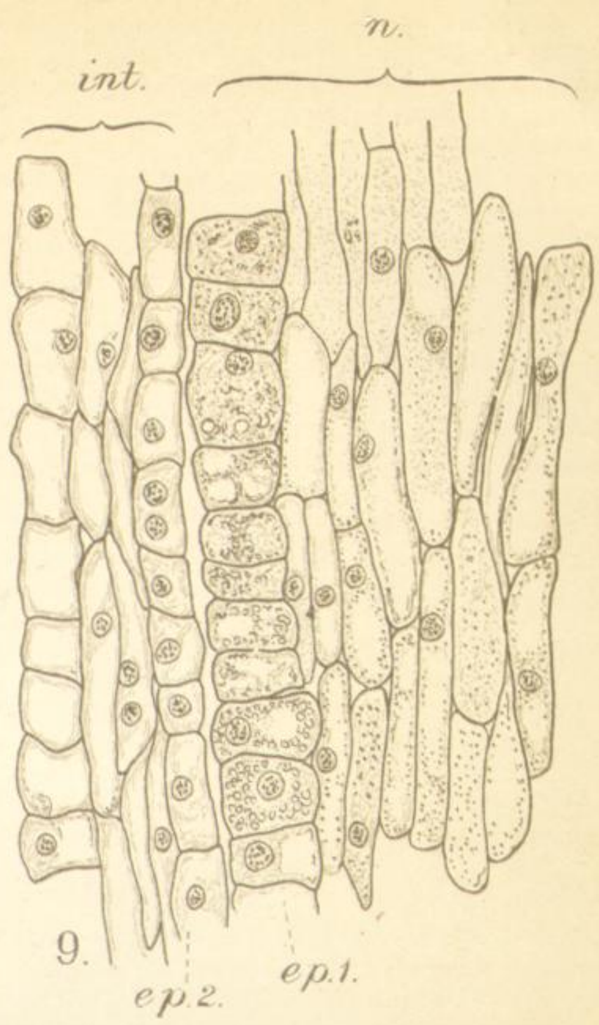

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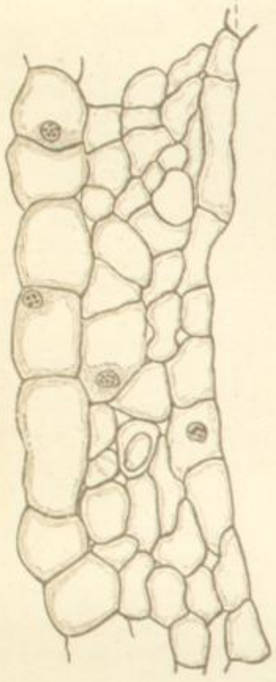

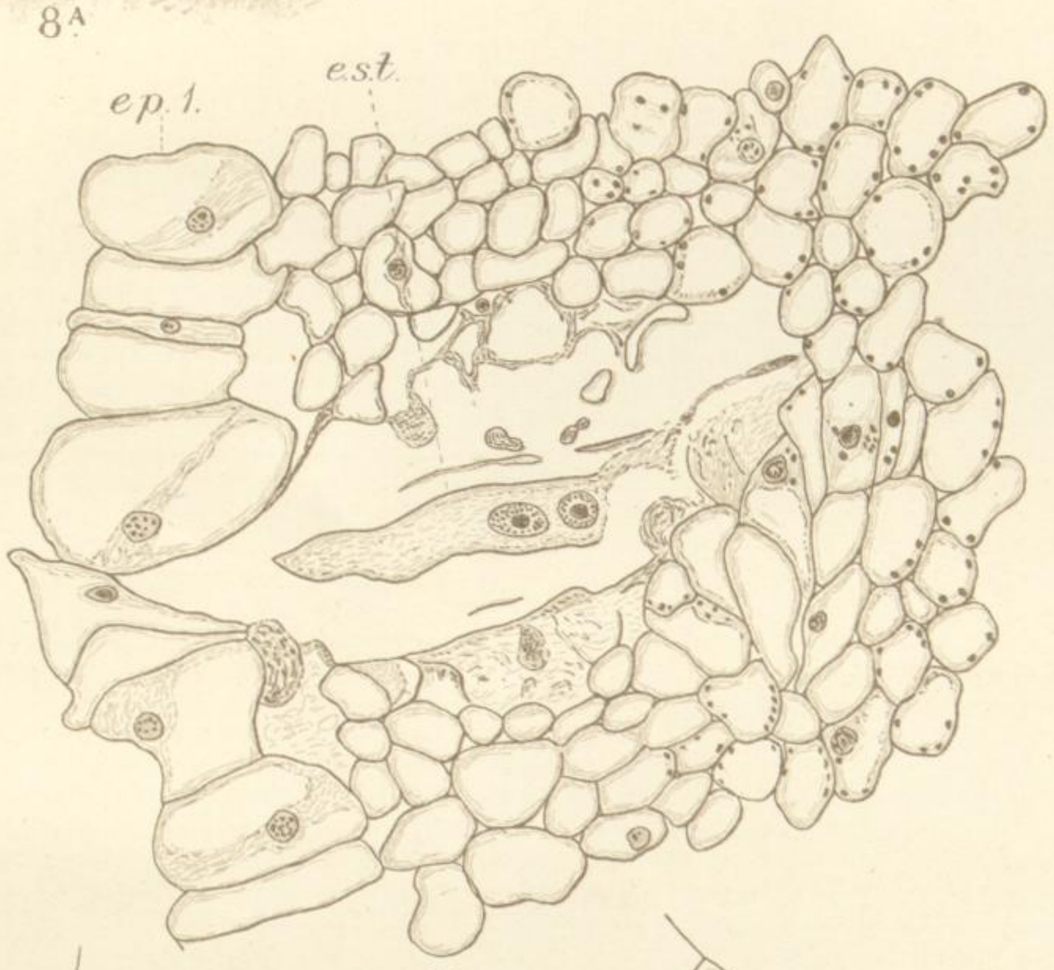

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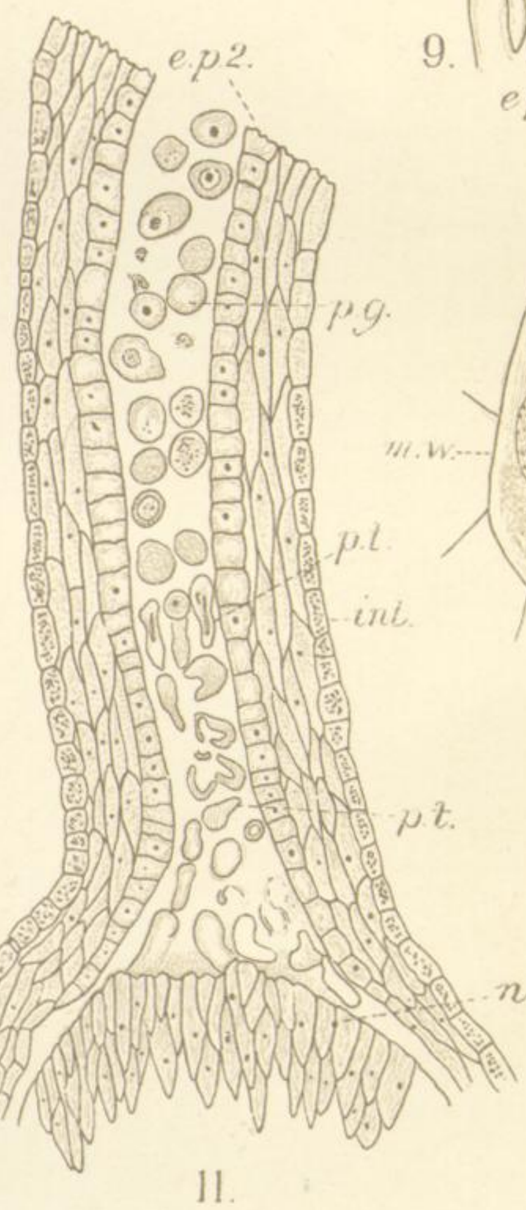

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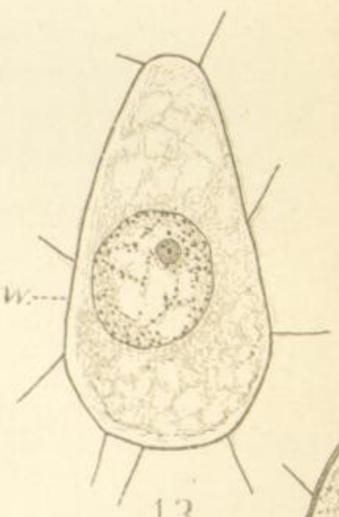

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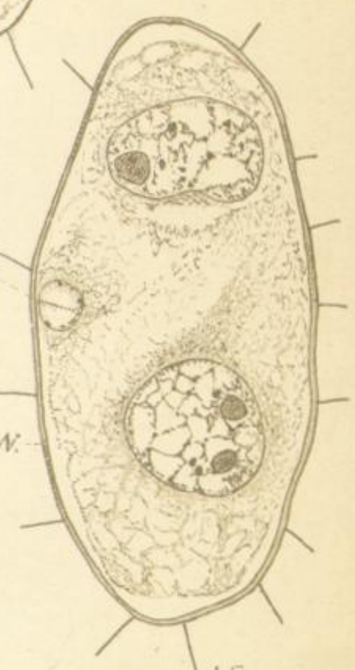

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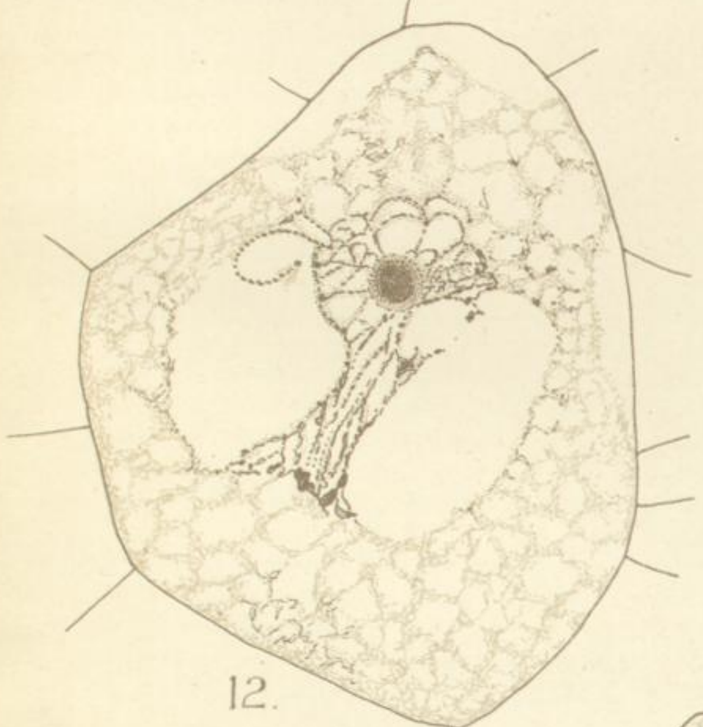

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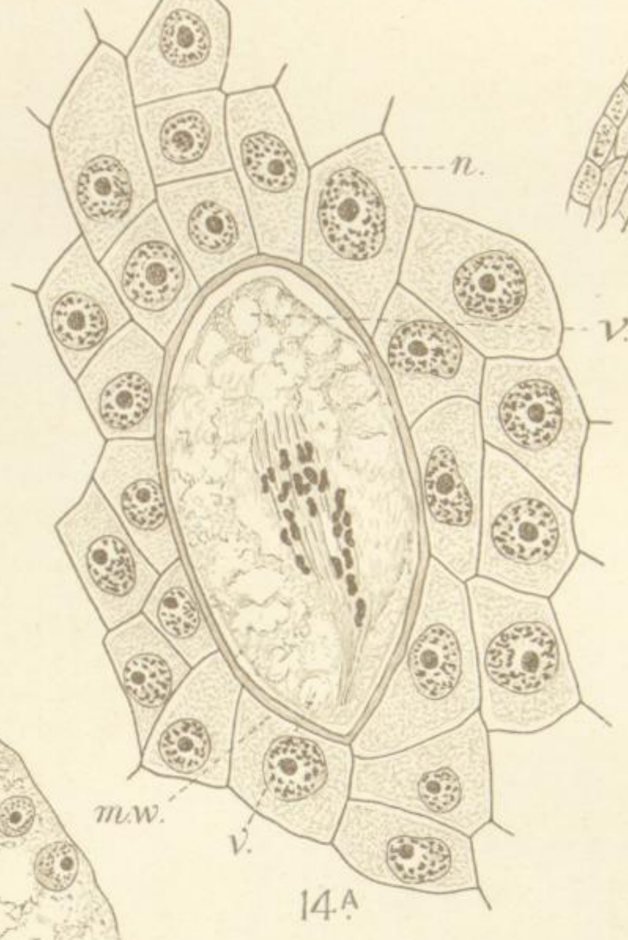

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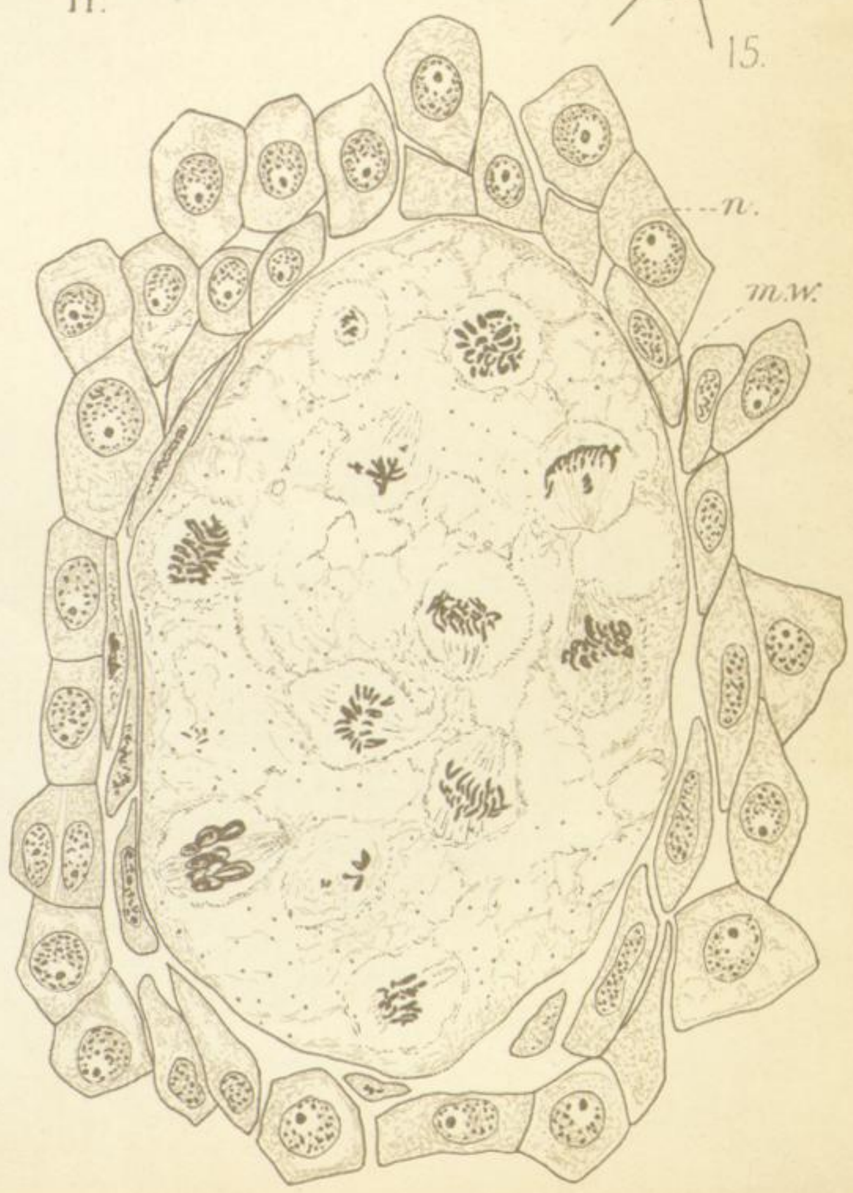

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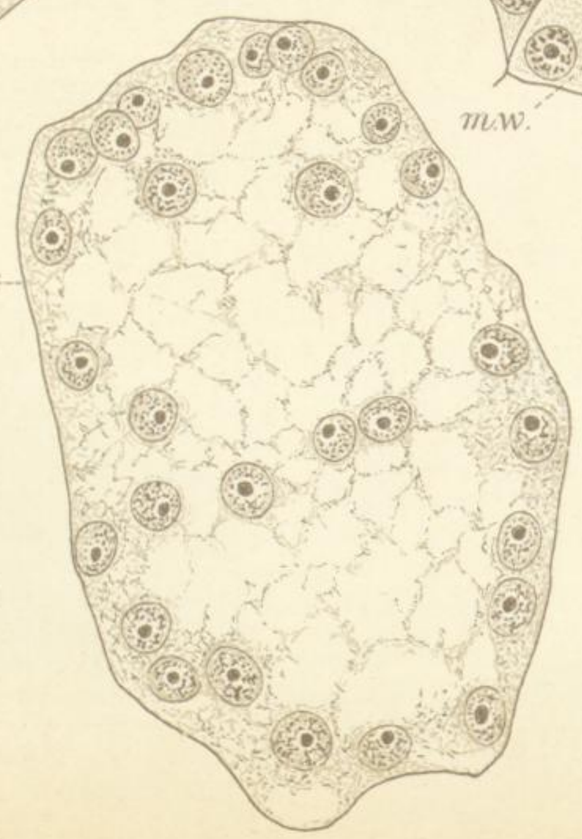

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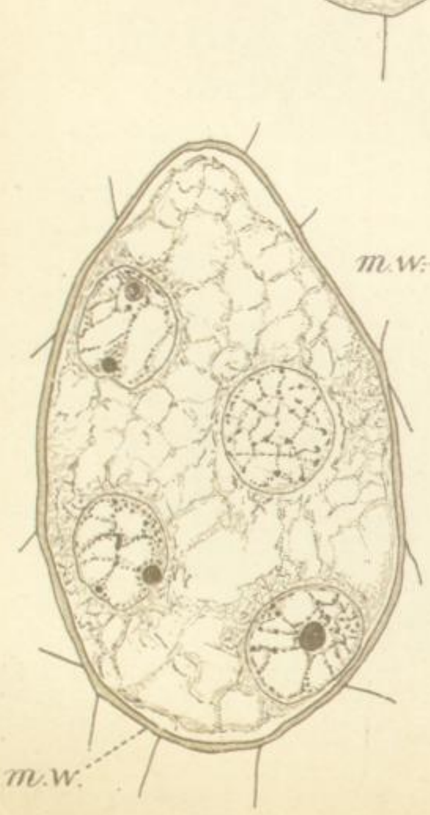

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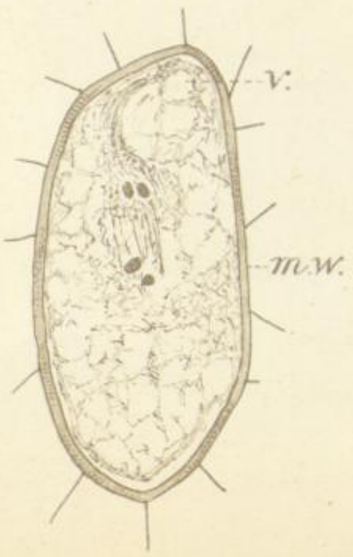

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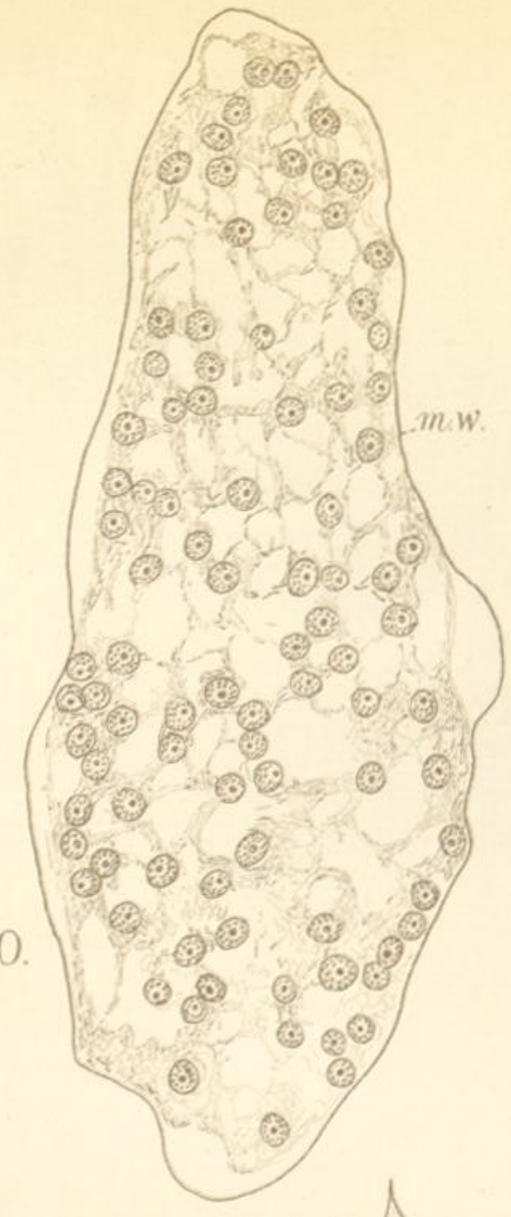

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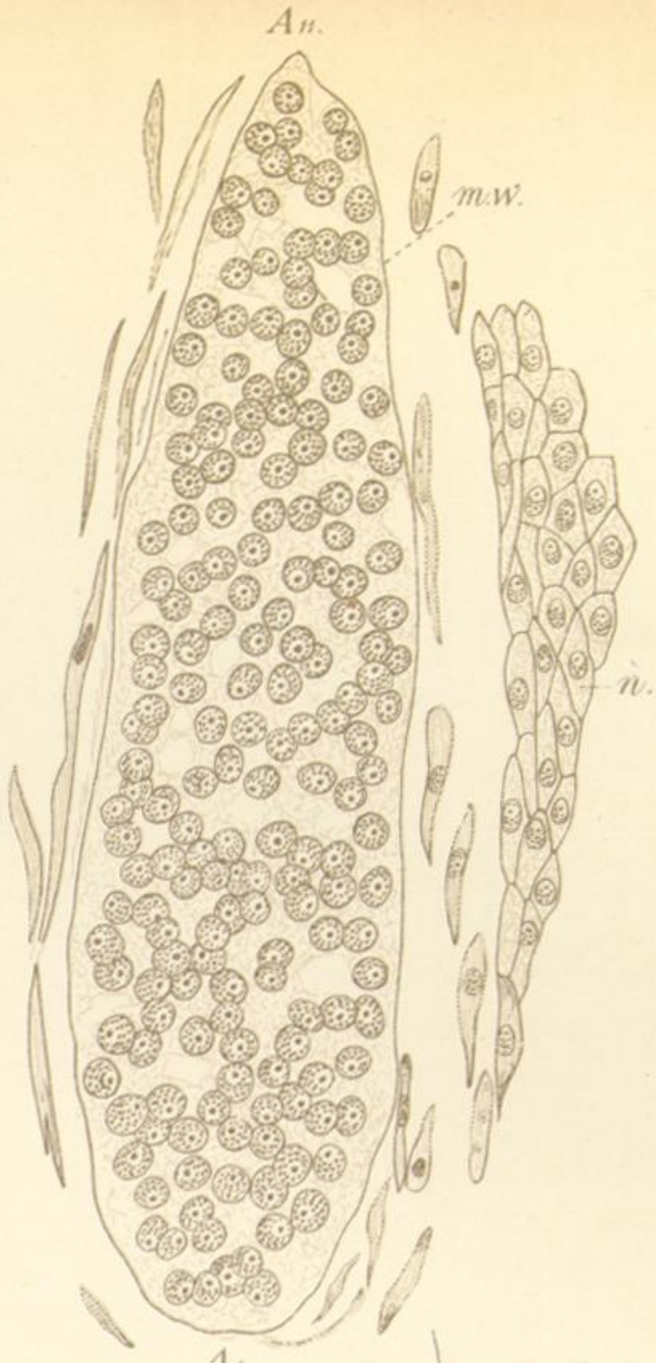

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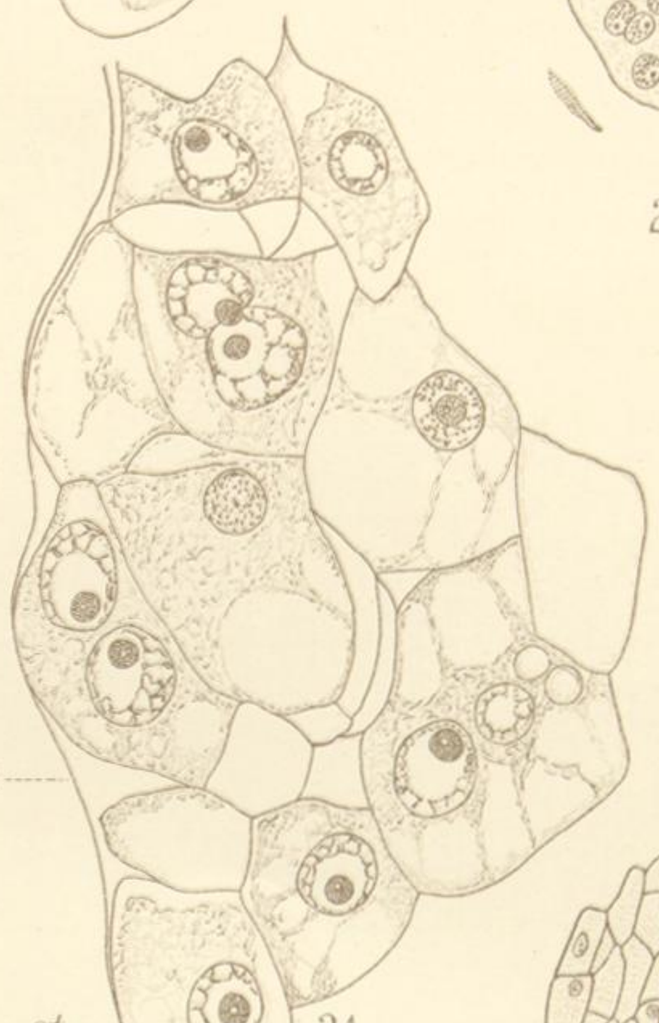

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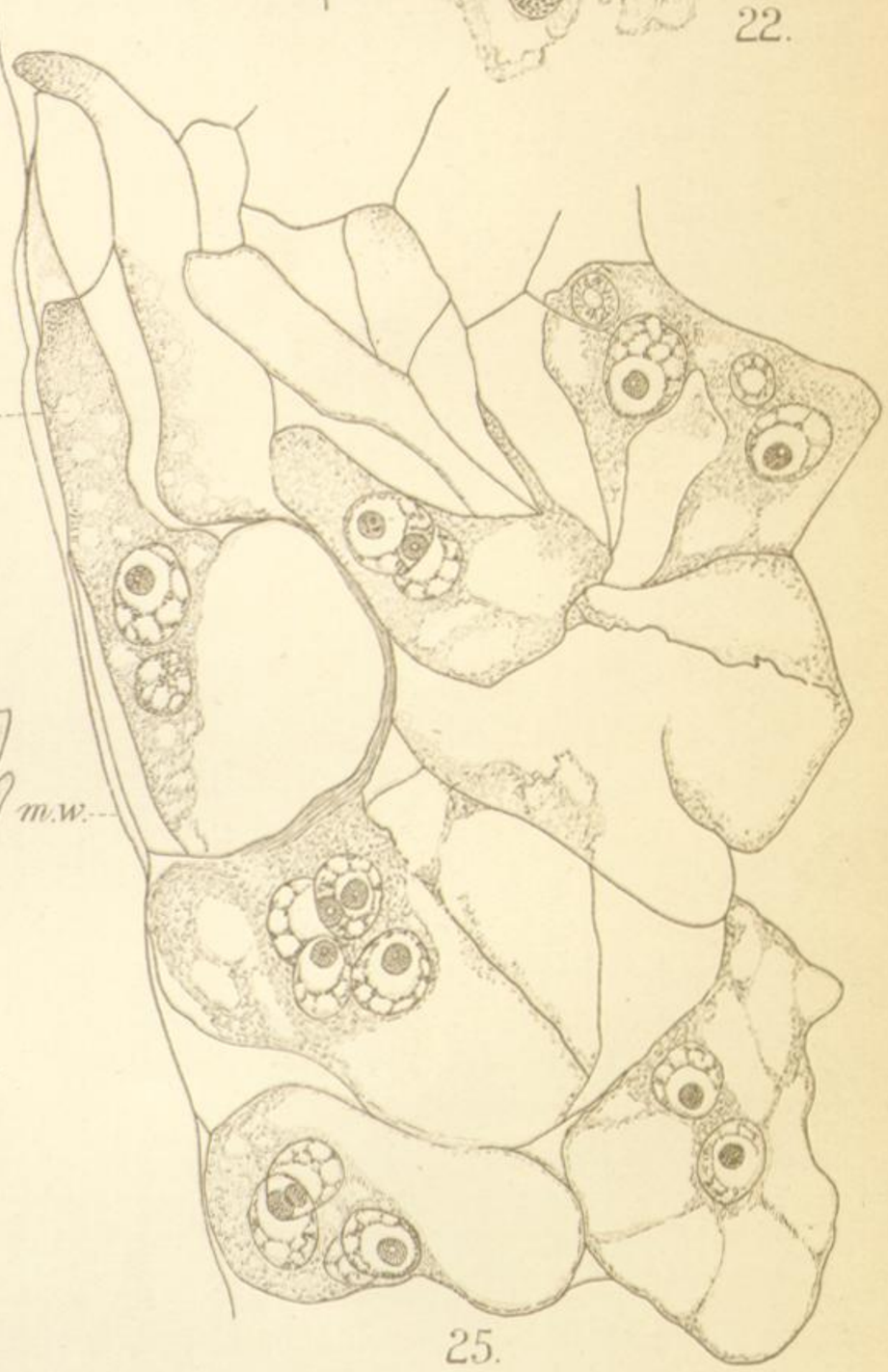

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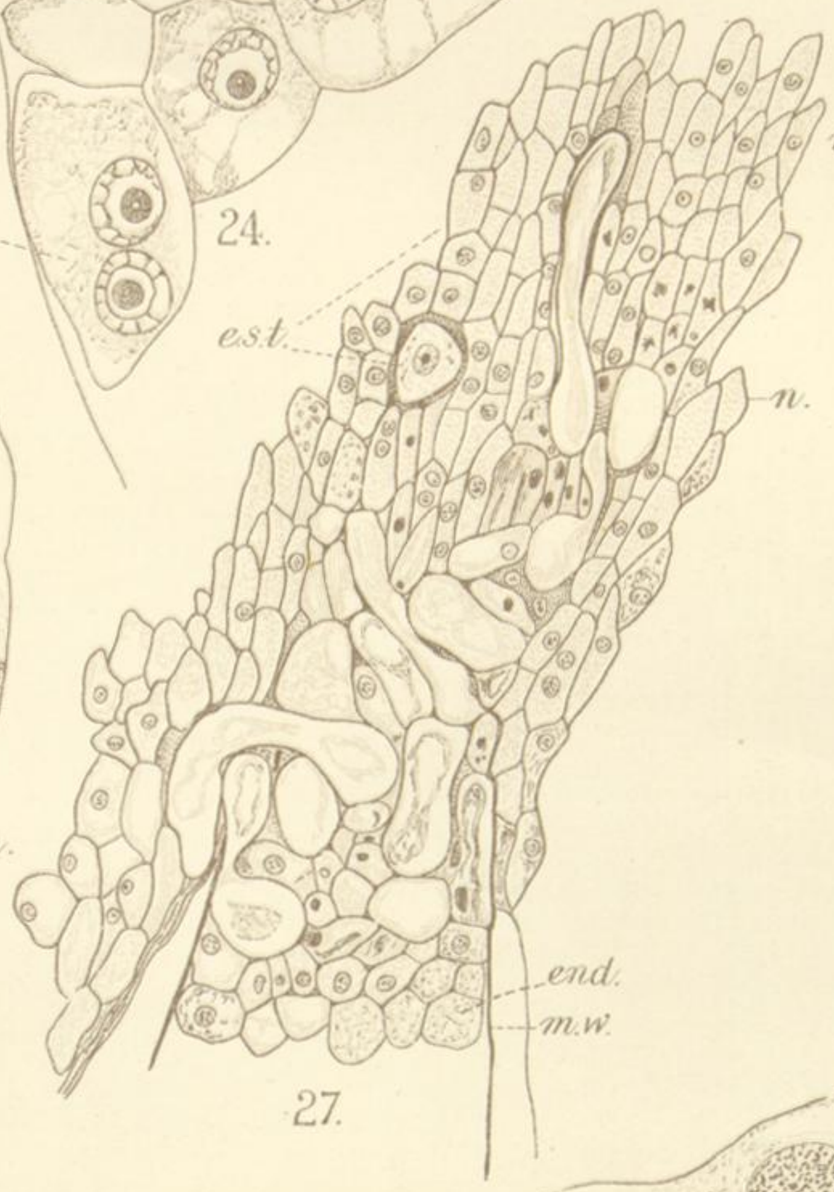

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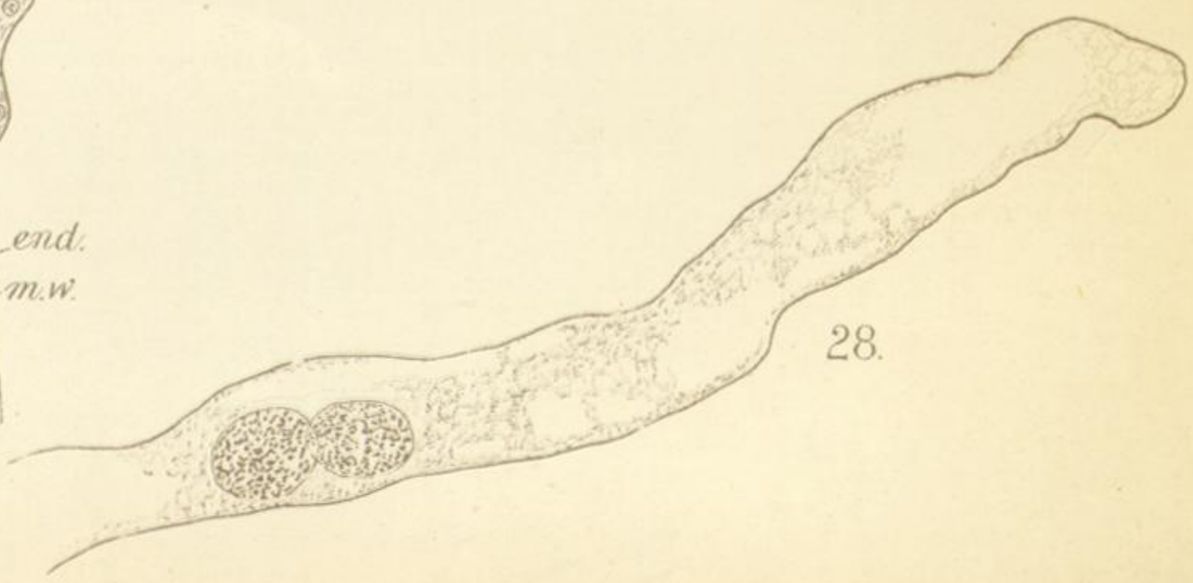

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29.

30B

st.

n.

est.

30A

31.

m.w.

32.

f.

a.

m.w.

33.

f.

34.

m.w.

f.

35B

35A

35C

m.w.

35D

f.

f.

f.

m.w.

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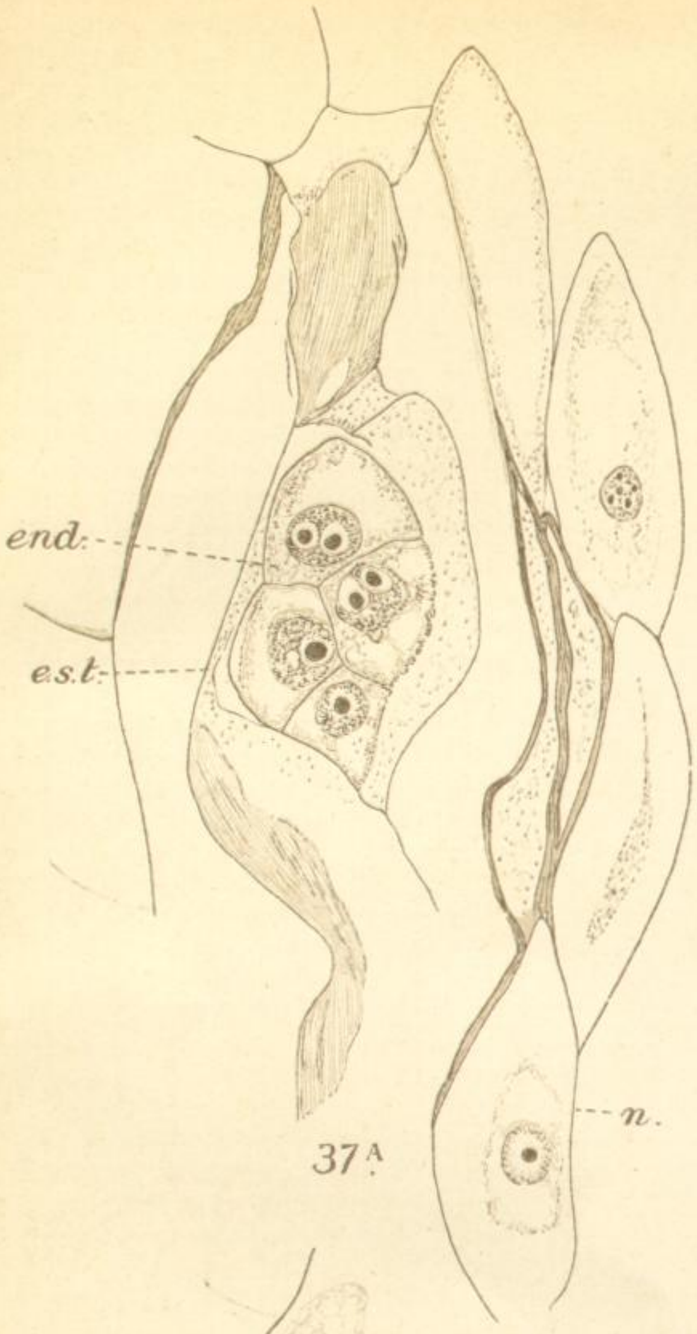

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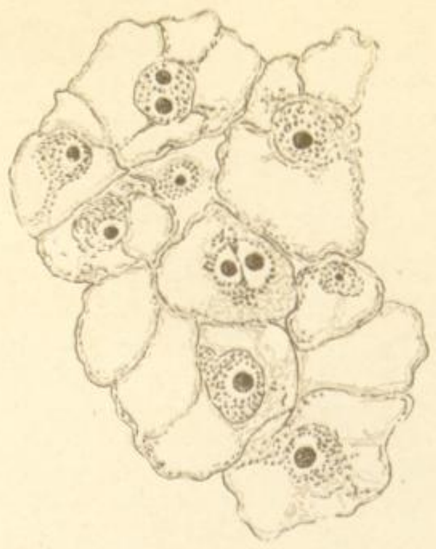

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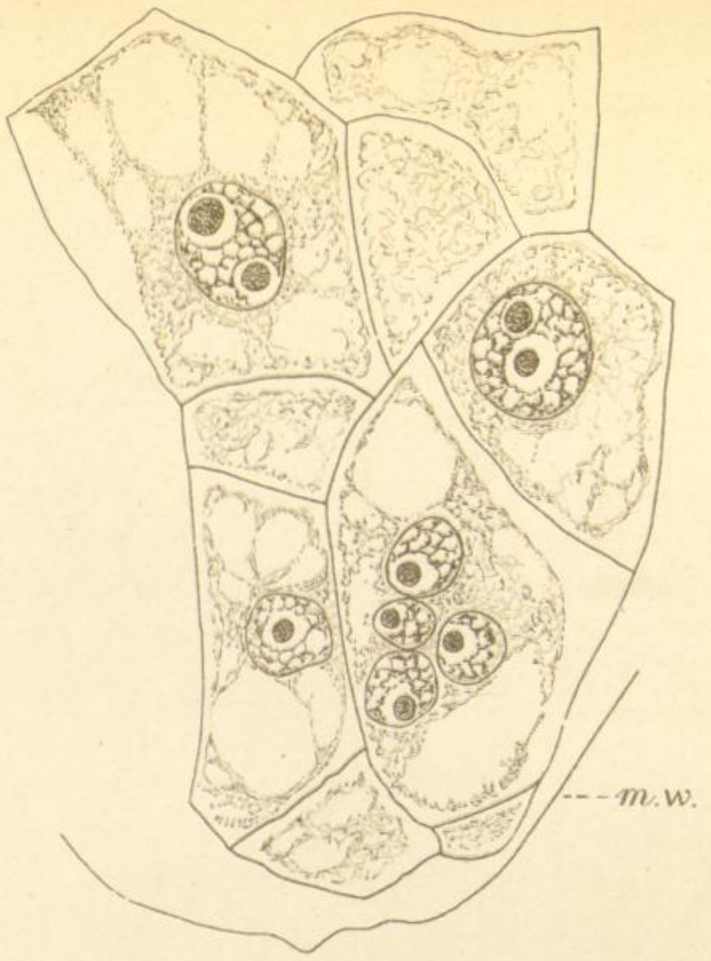

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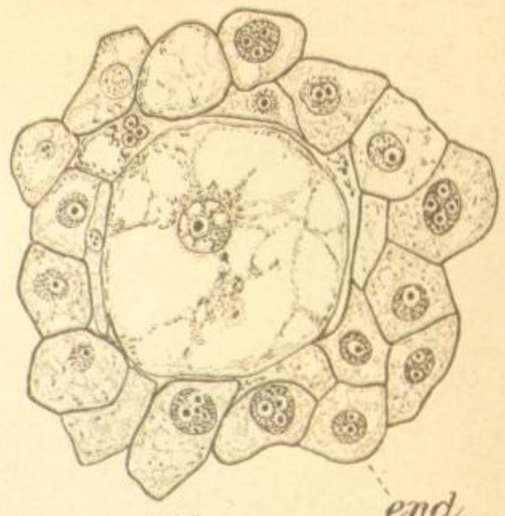

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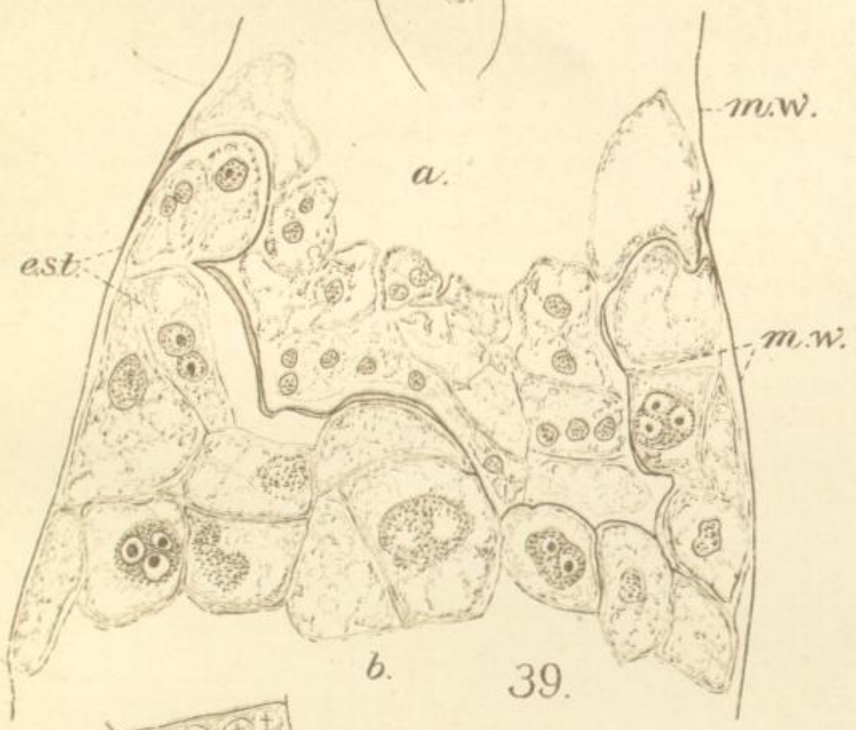

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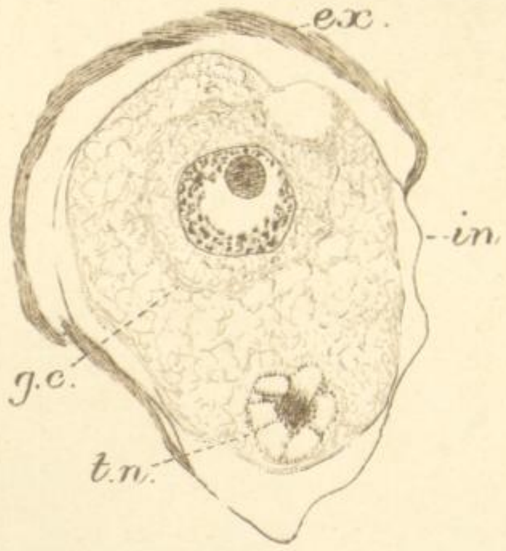

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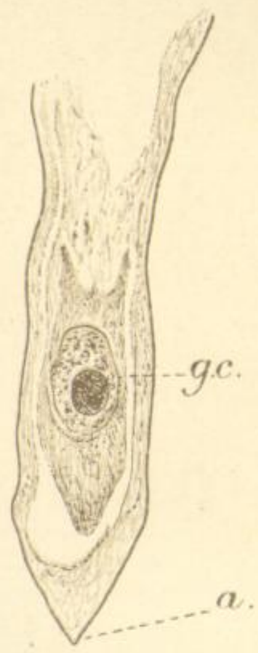

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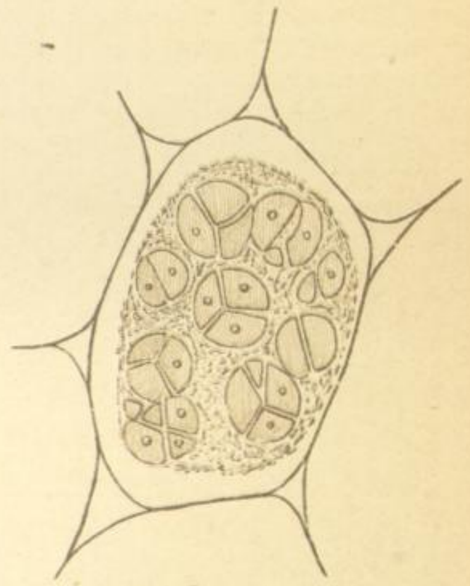

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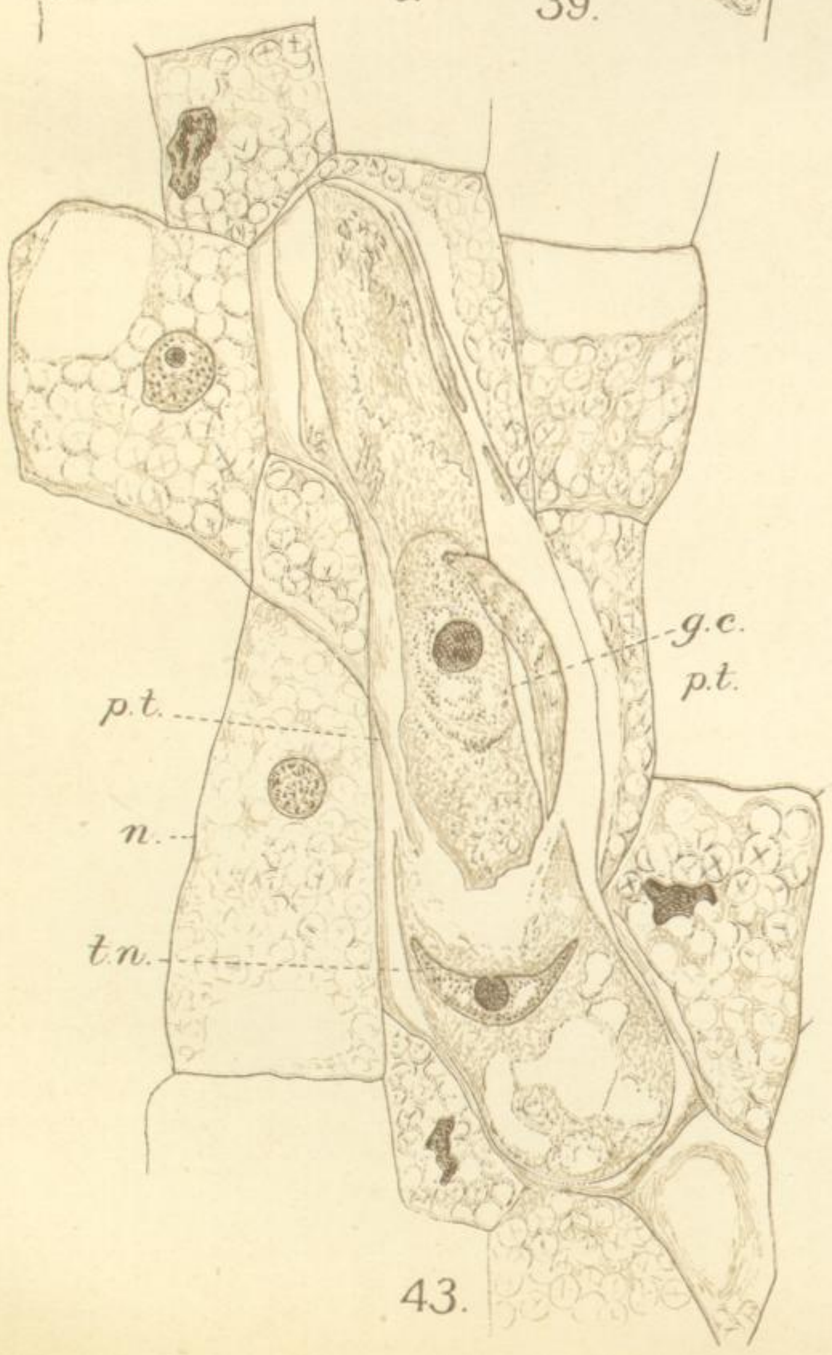

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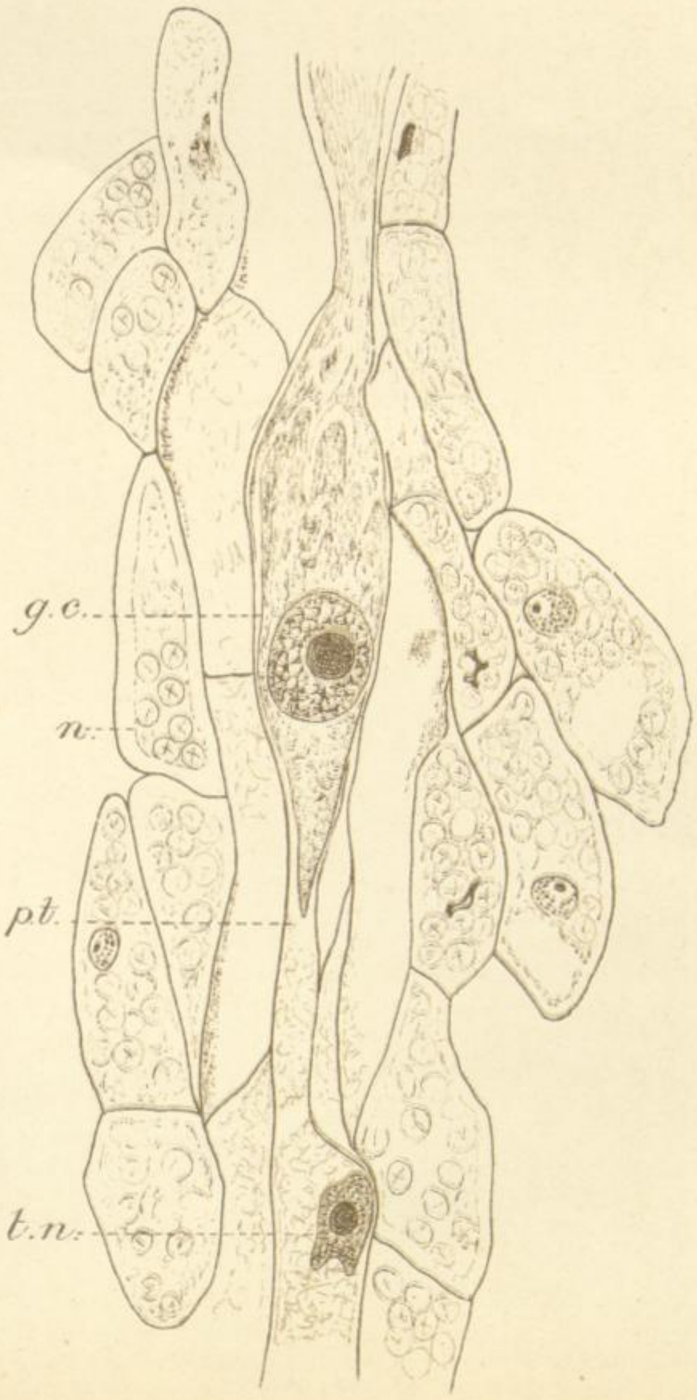

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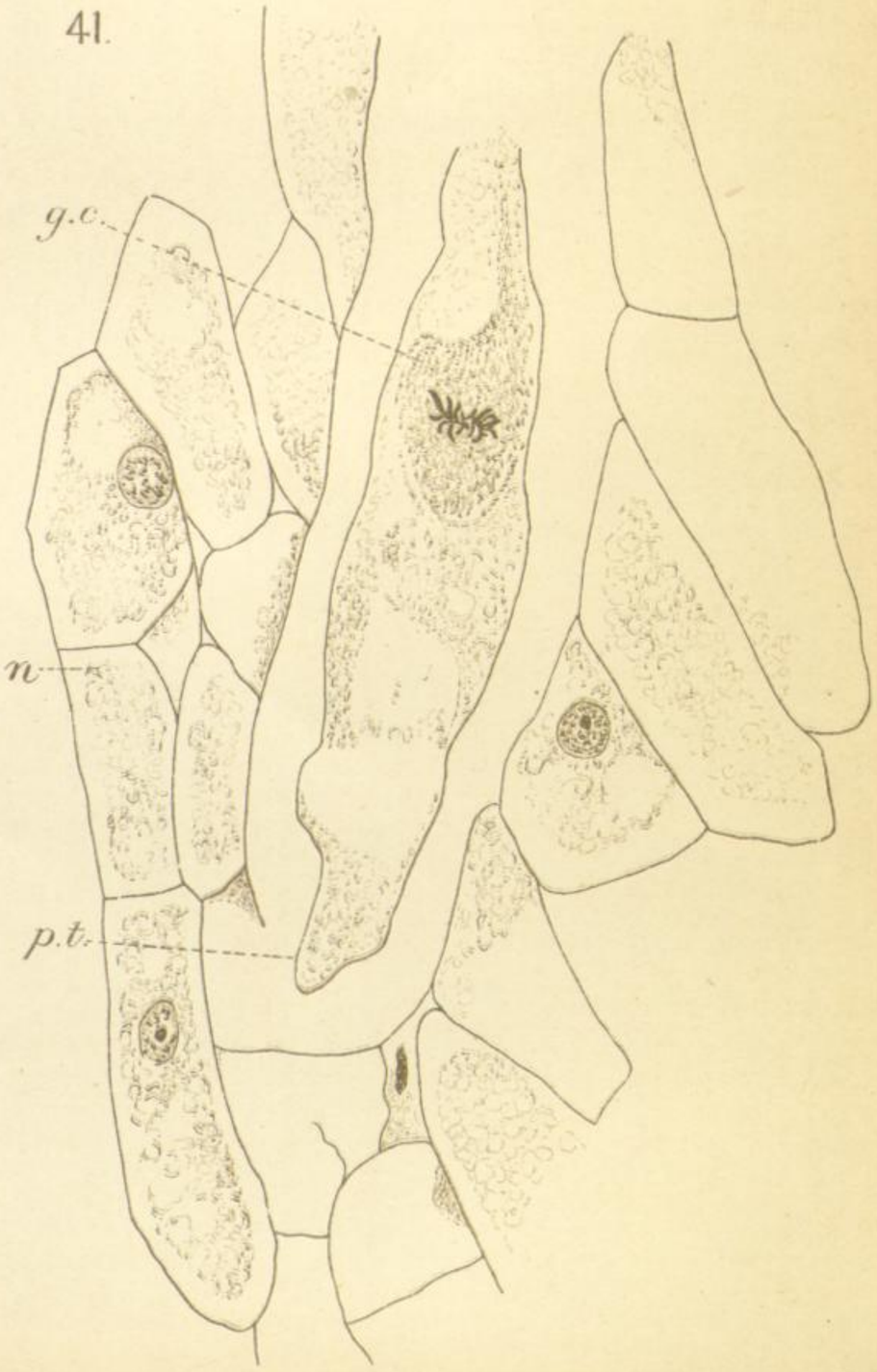

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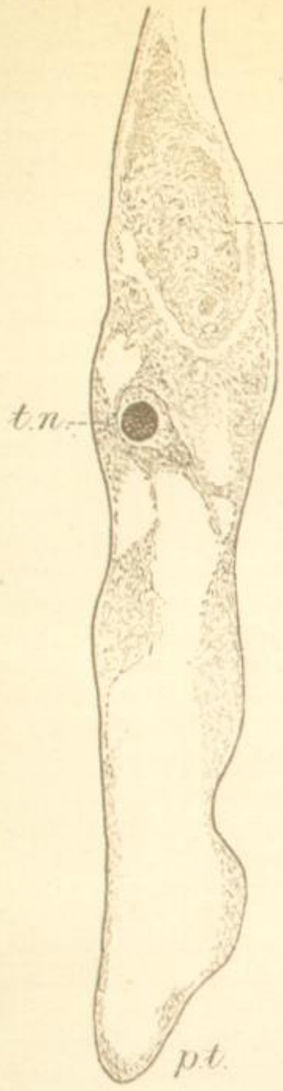

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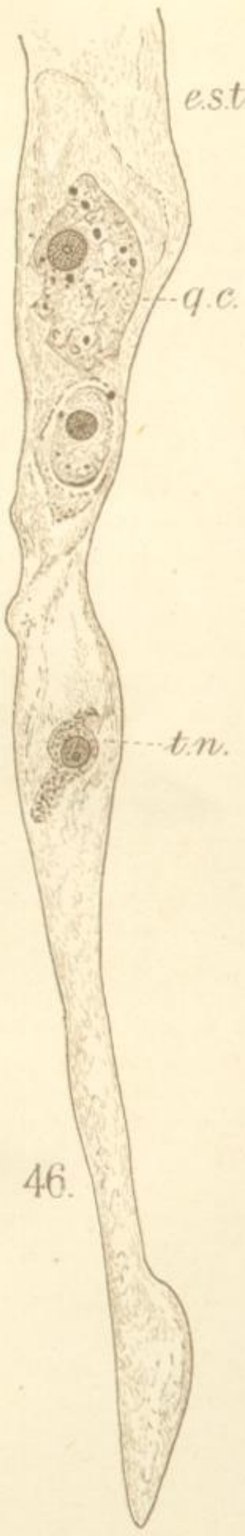

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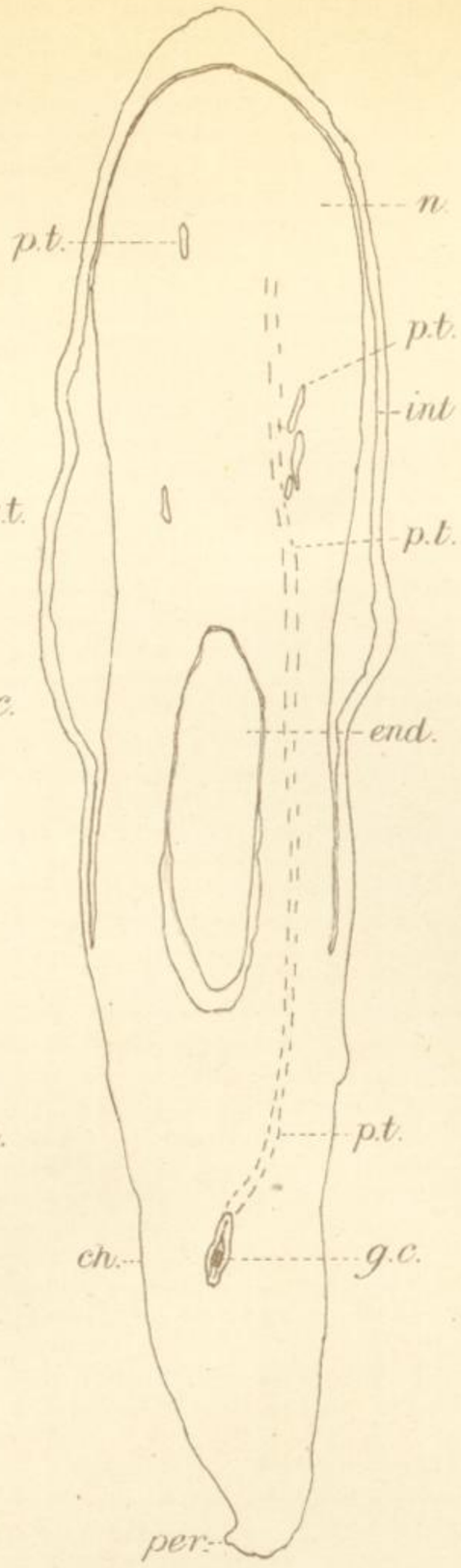

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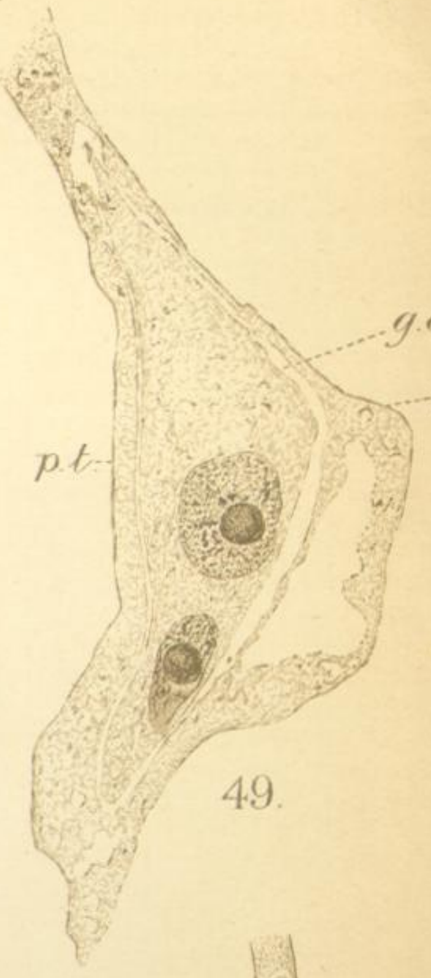

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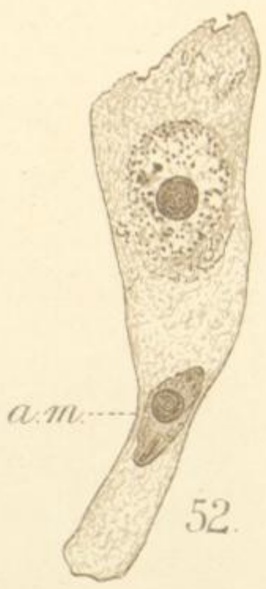

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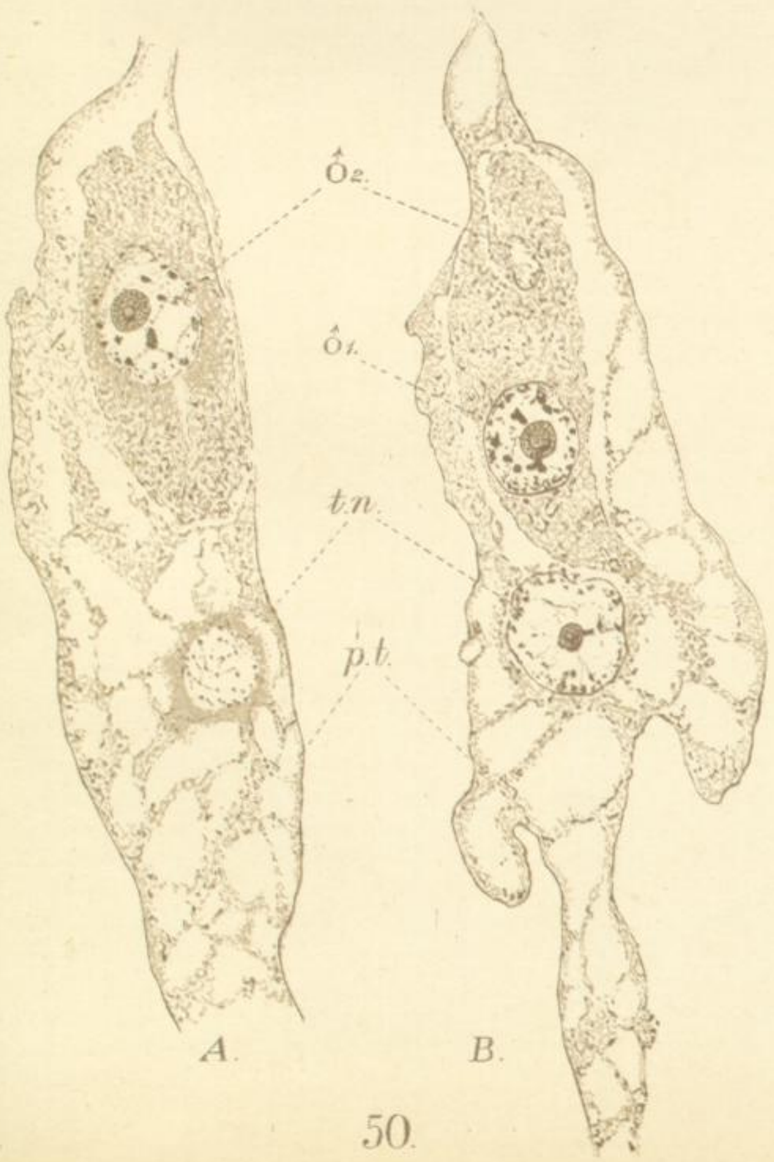

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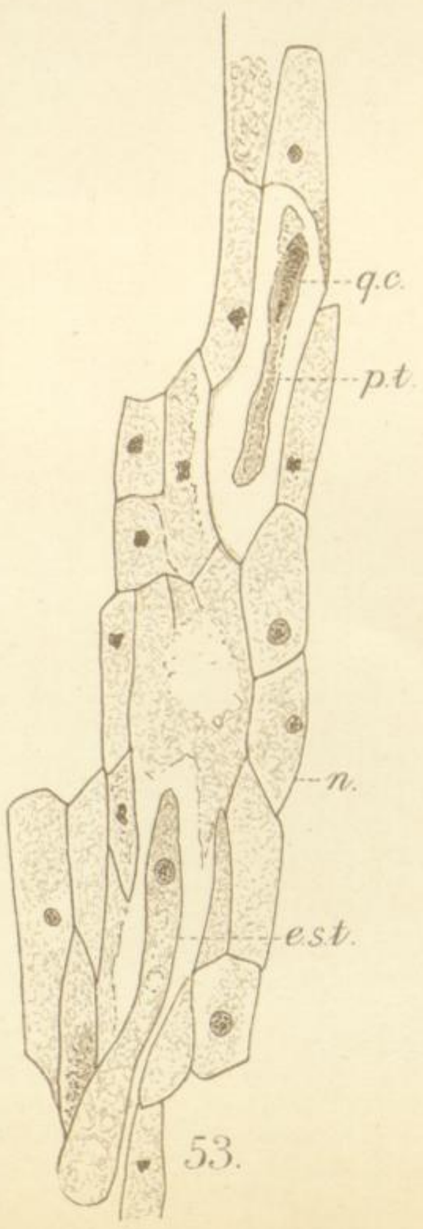

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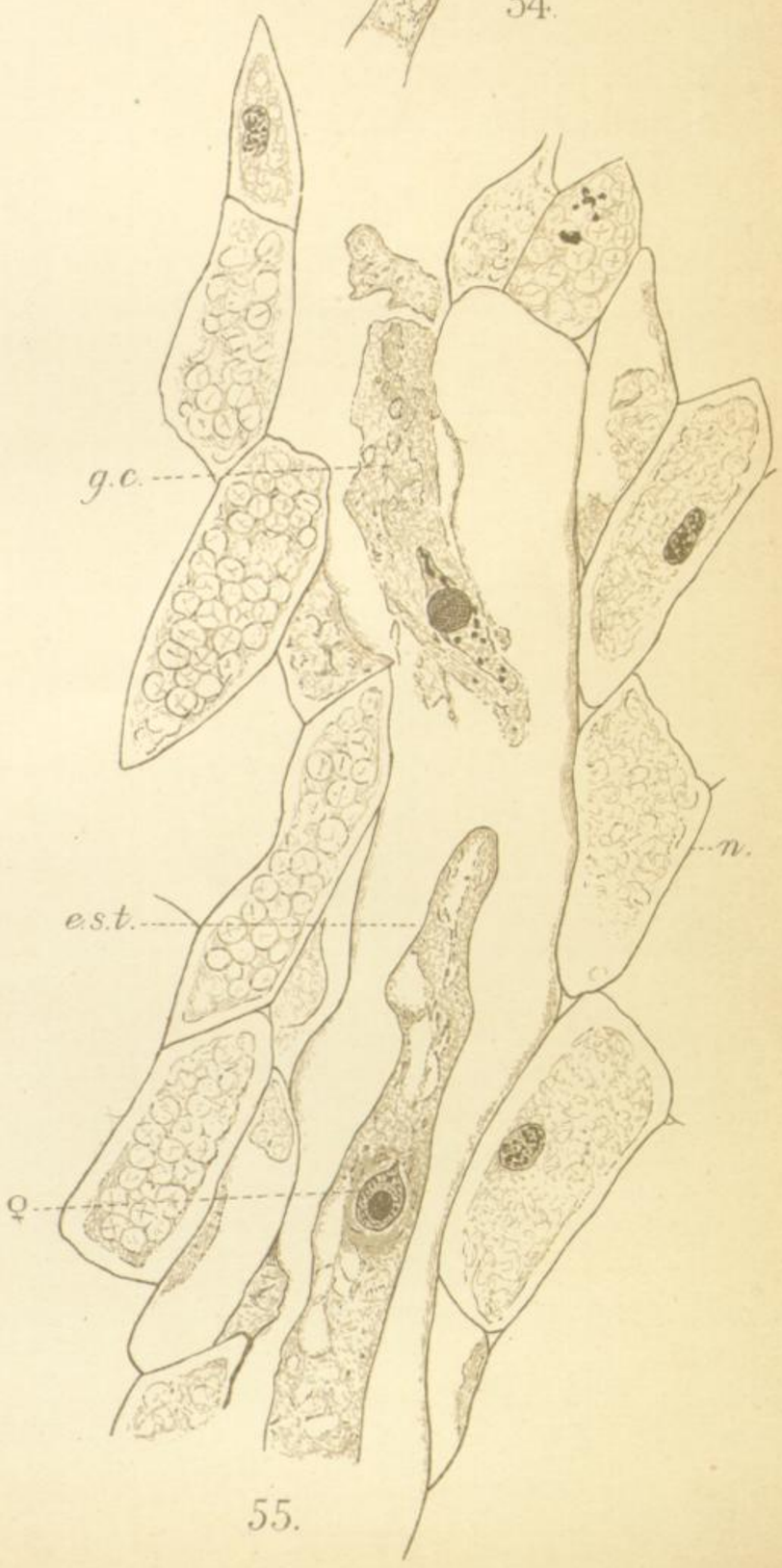

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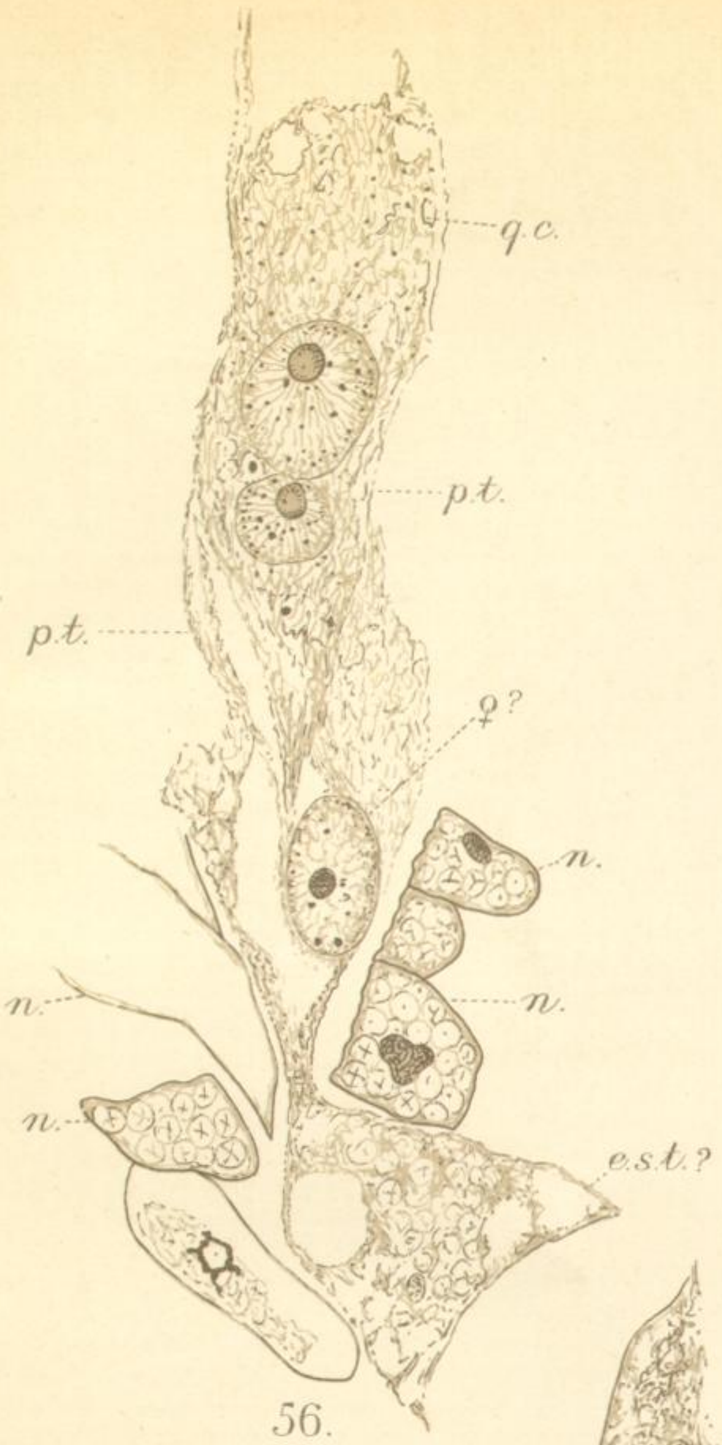

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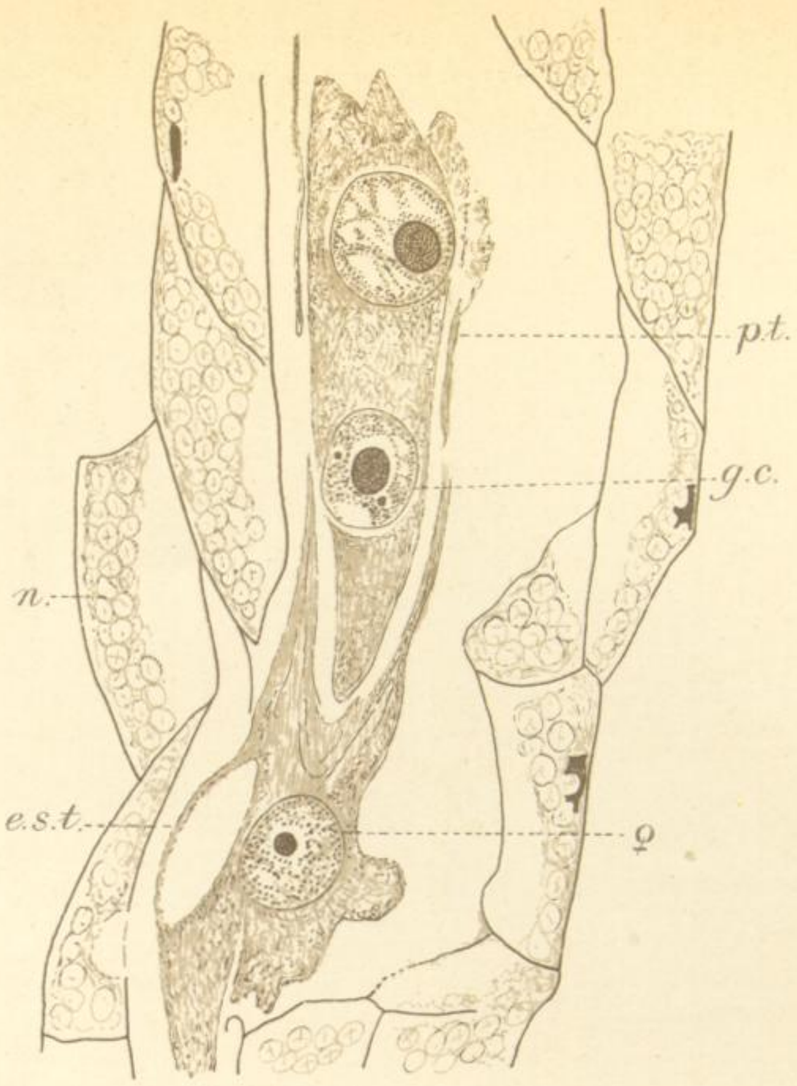

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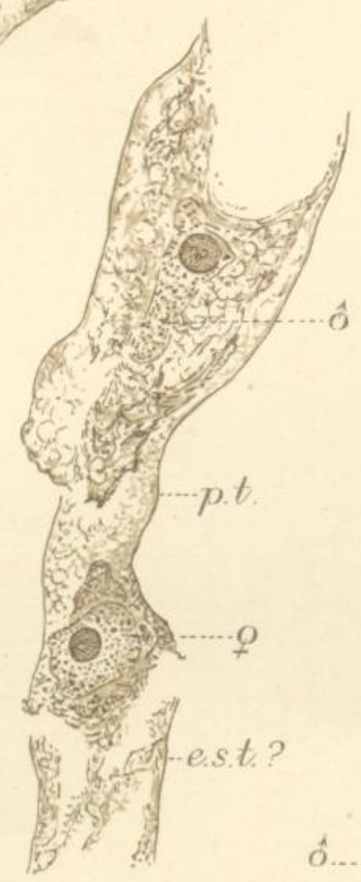

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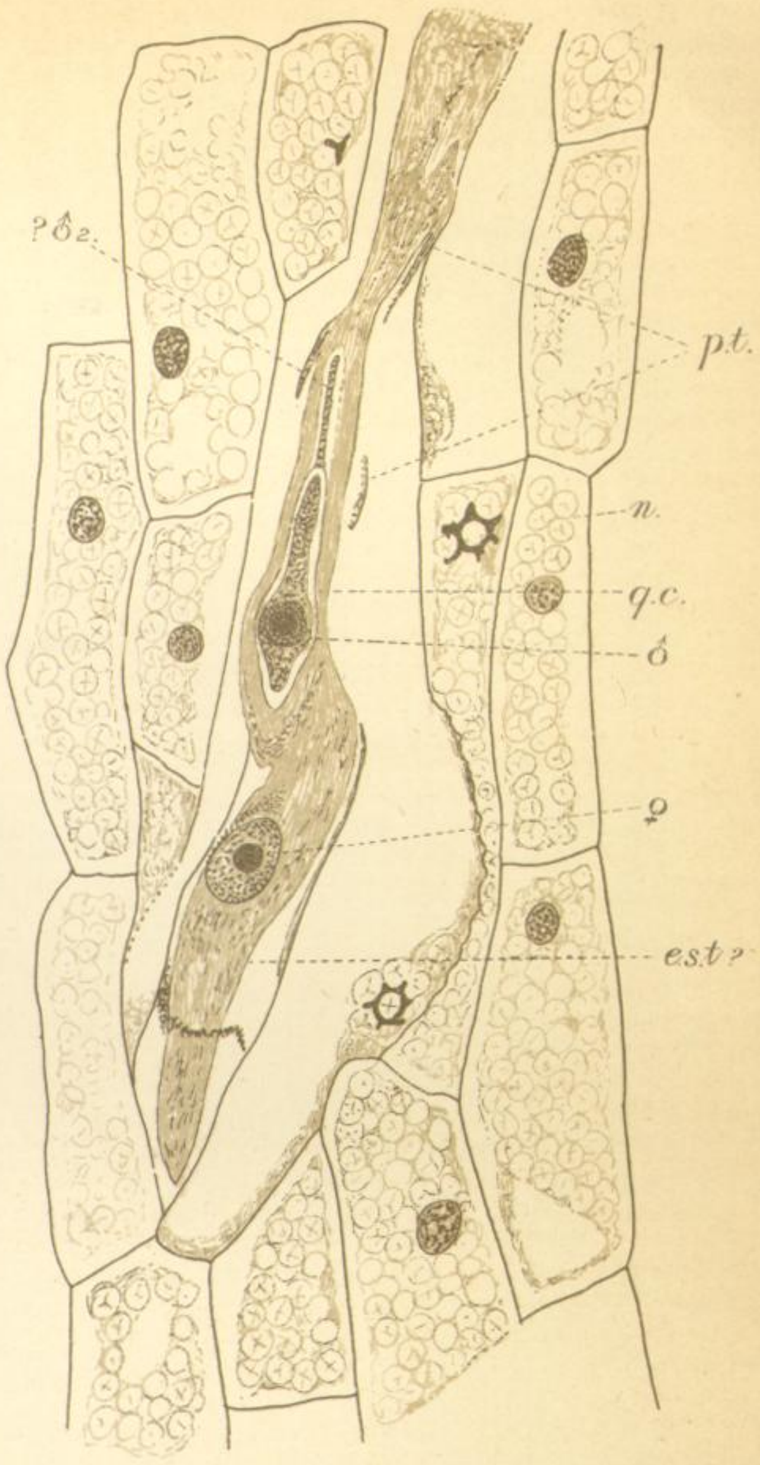

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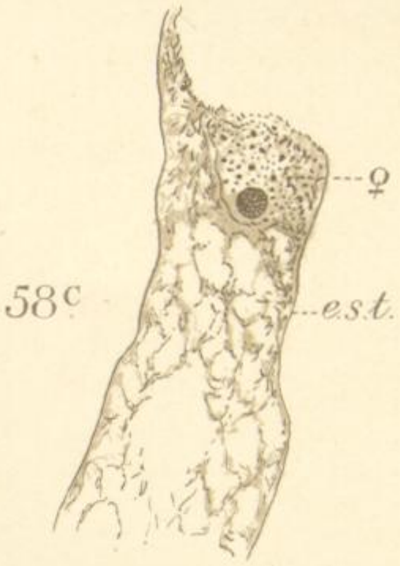

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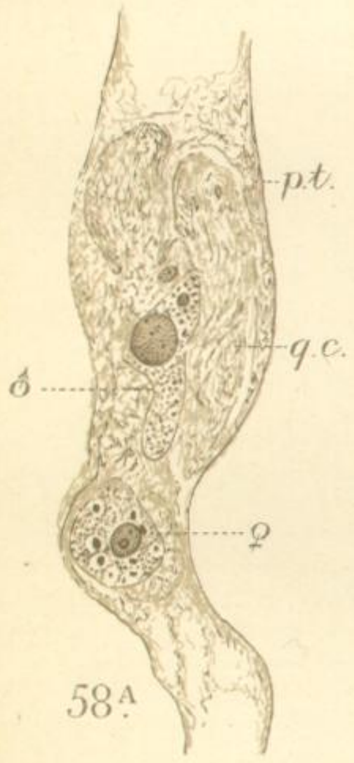

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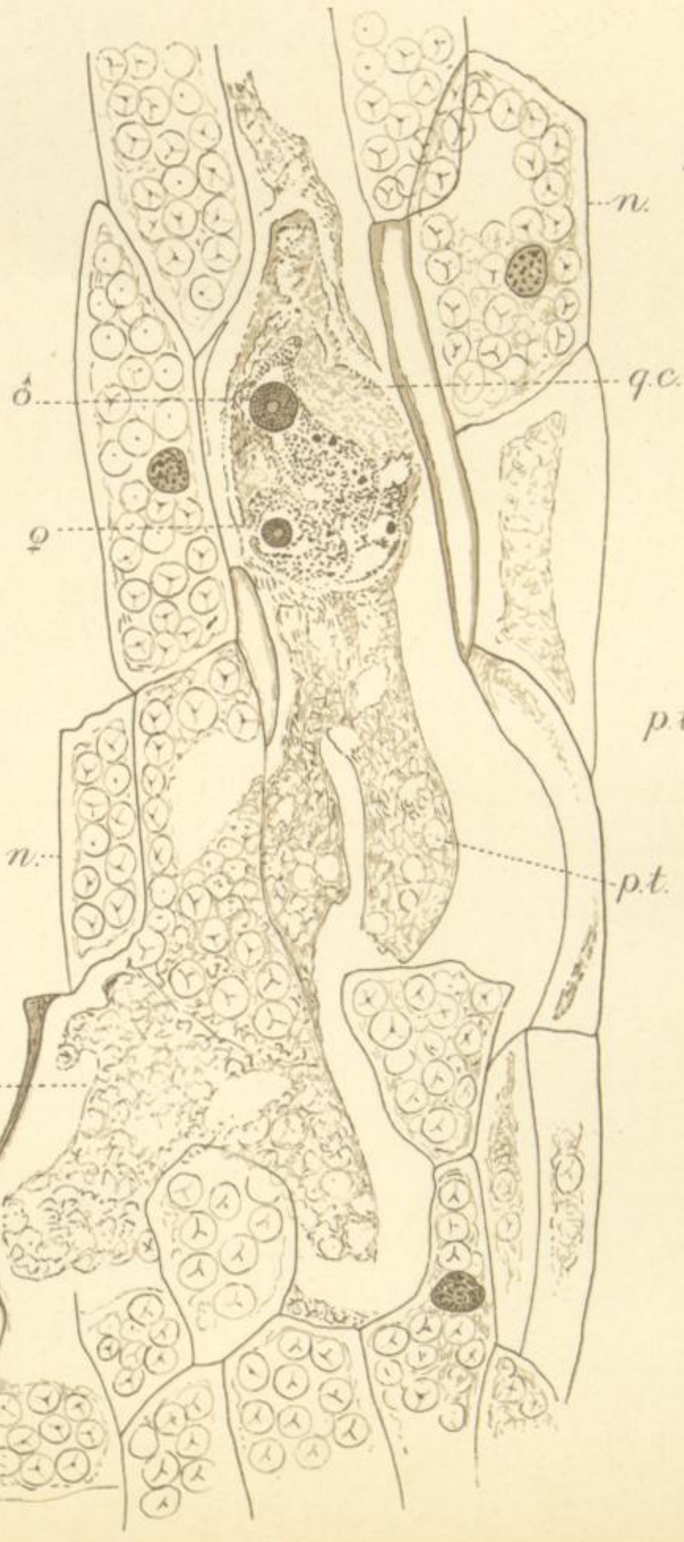

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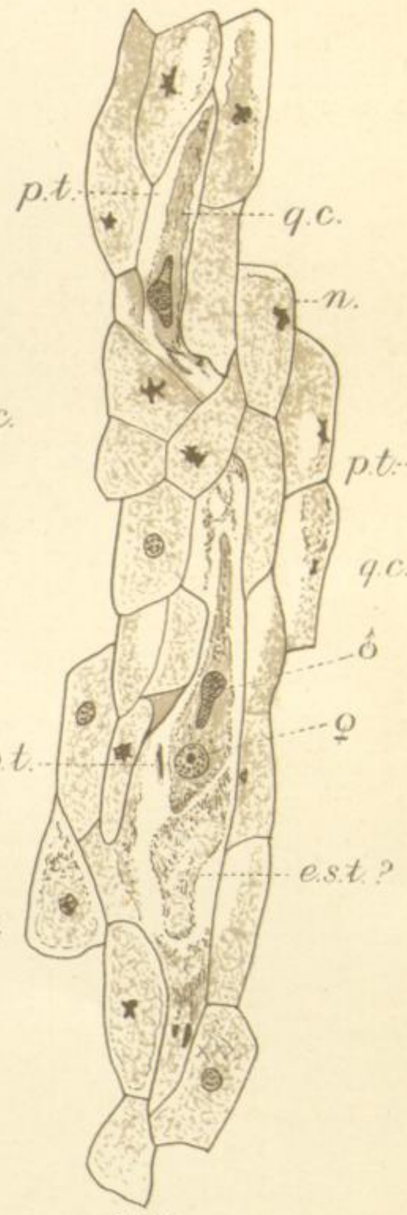

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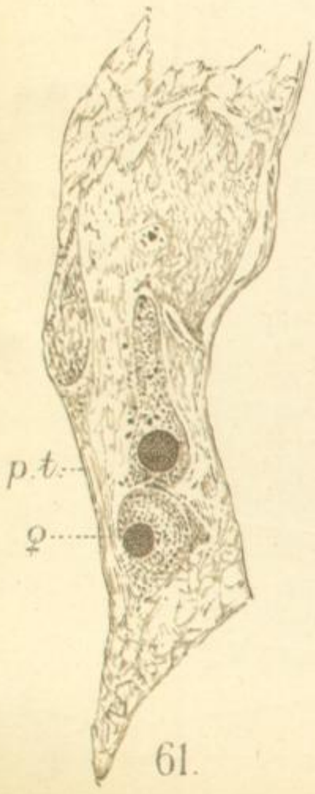

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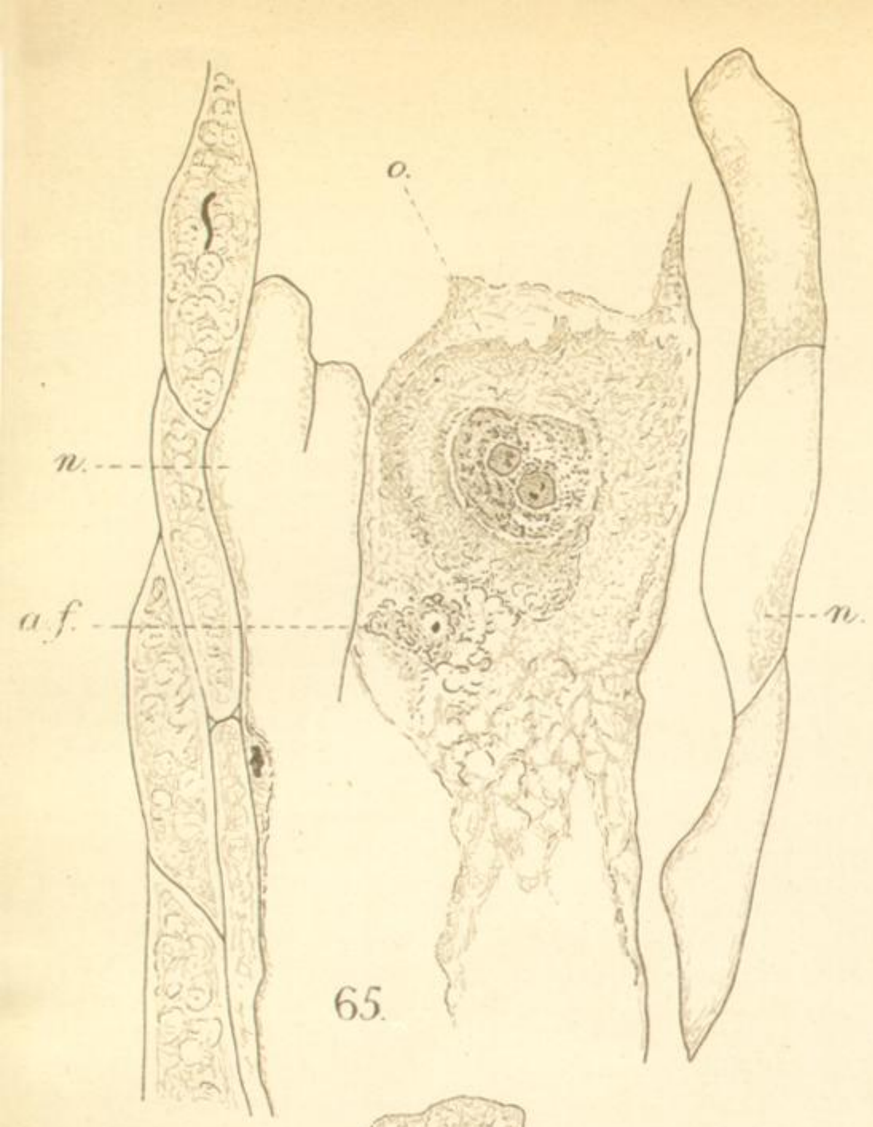

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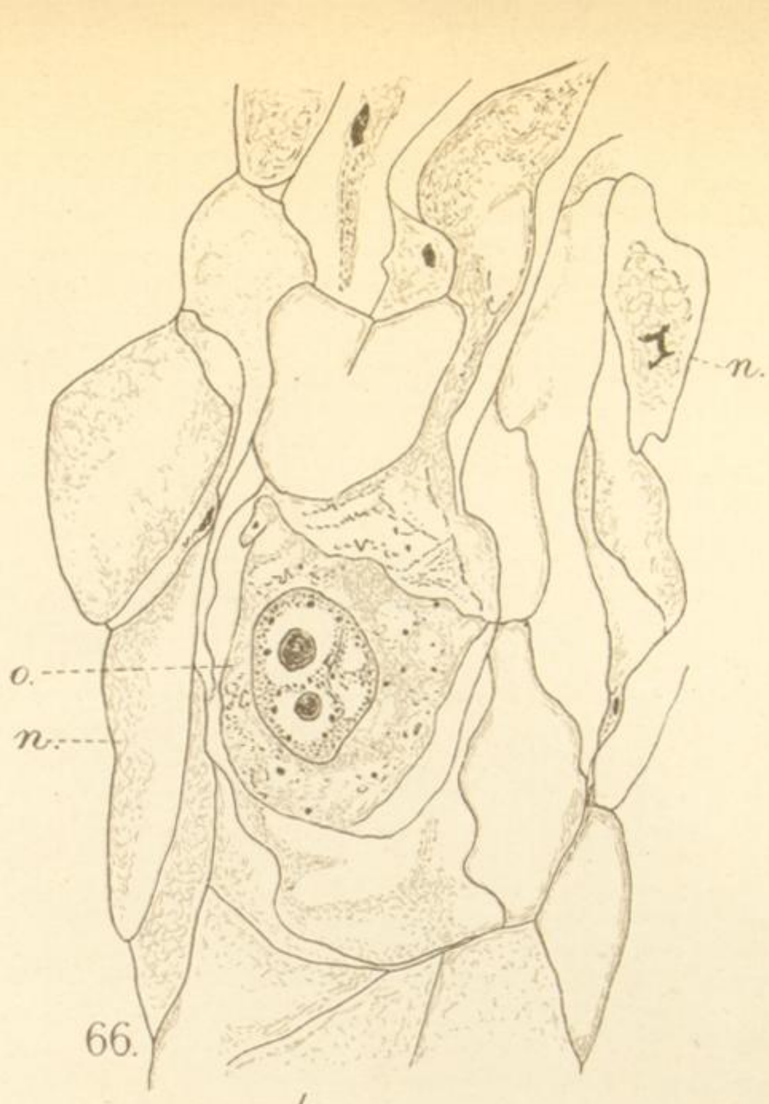

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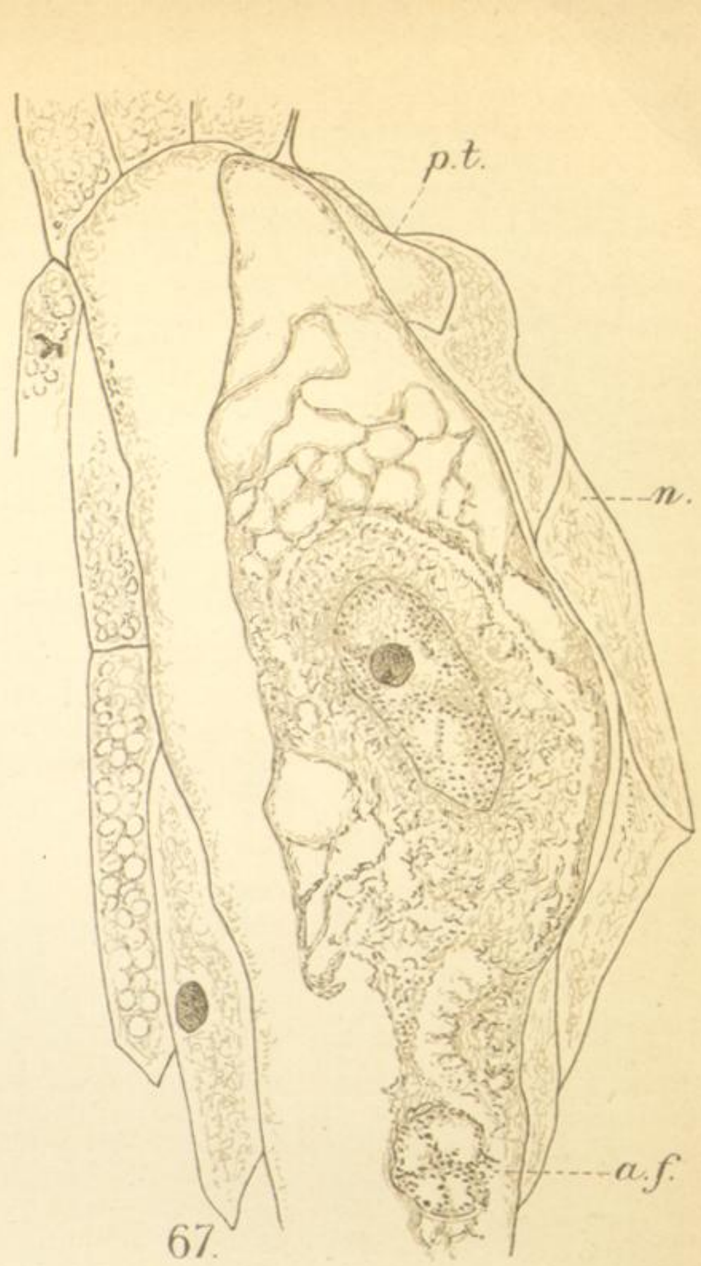

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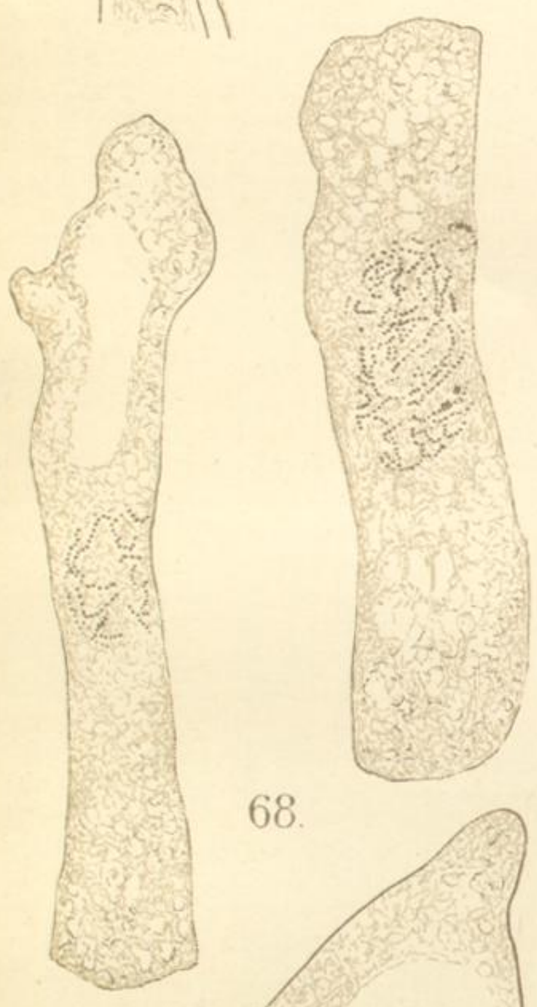

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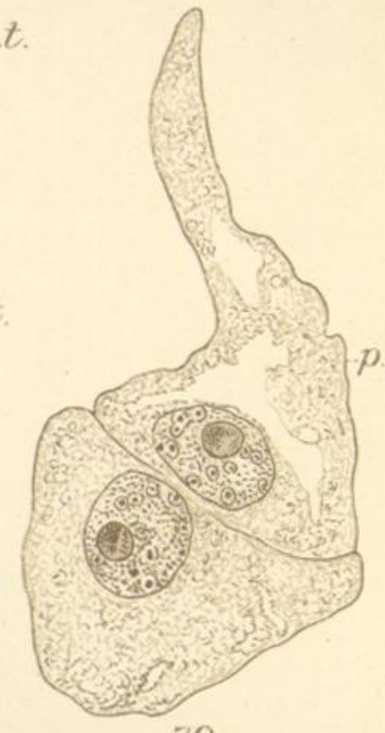

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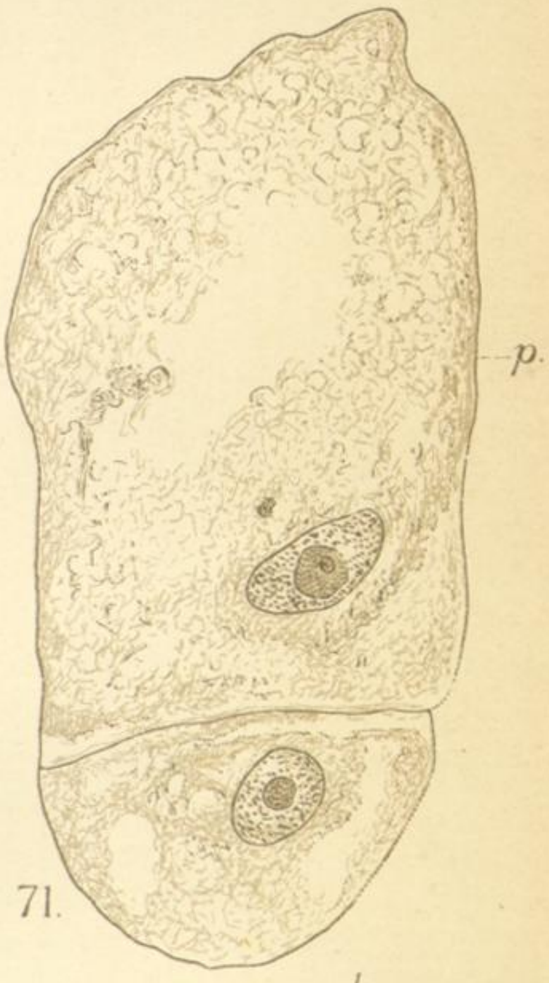

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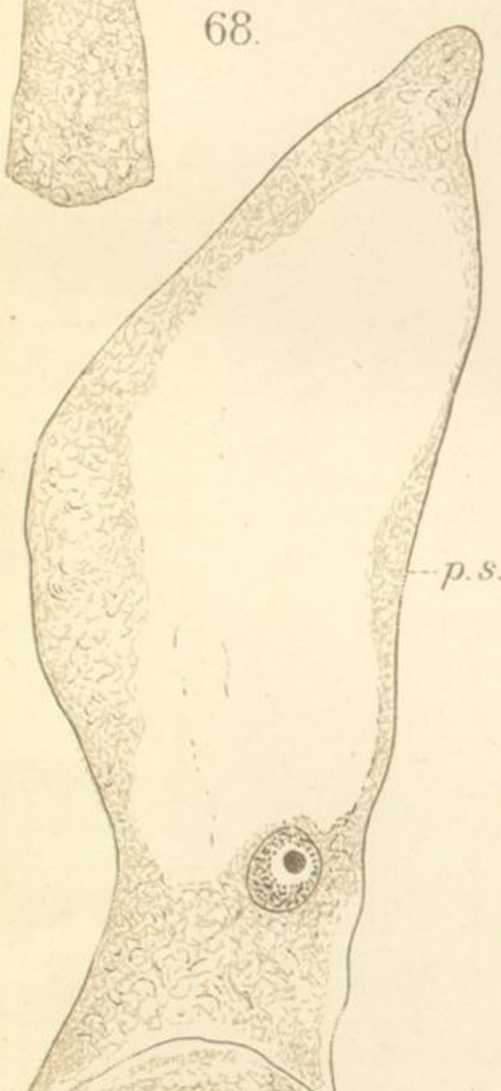

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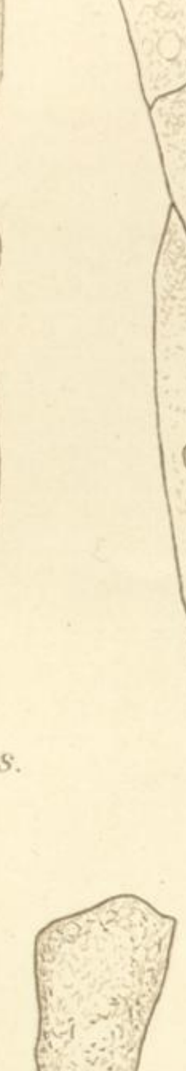

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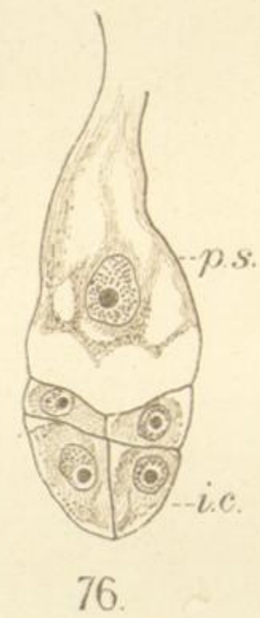

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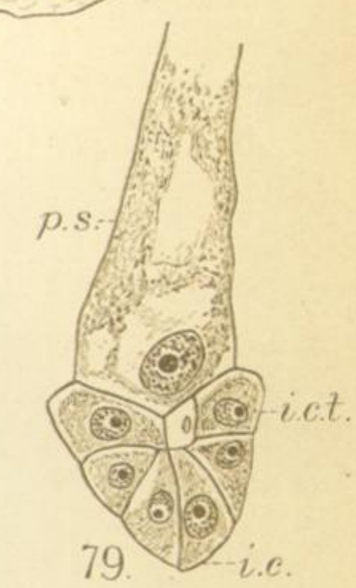

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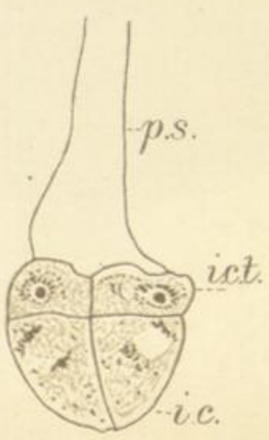

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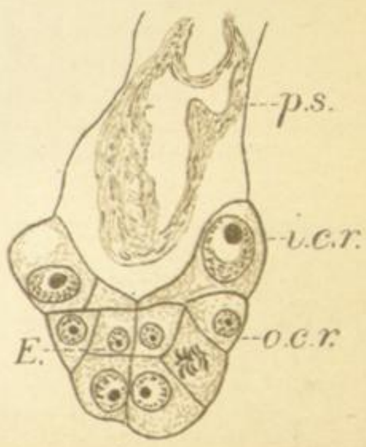

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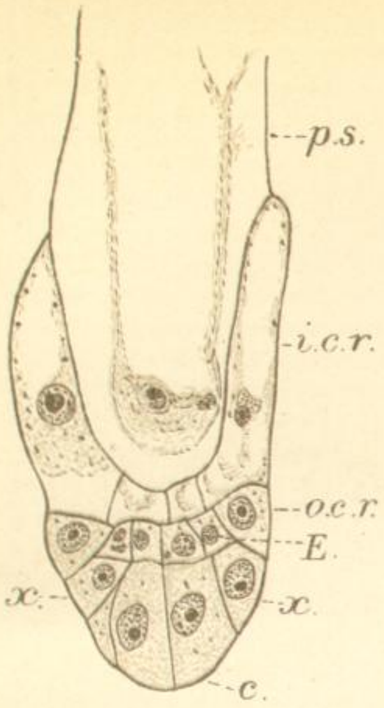

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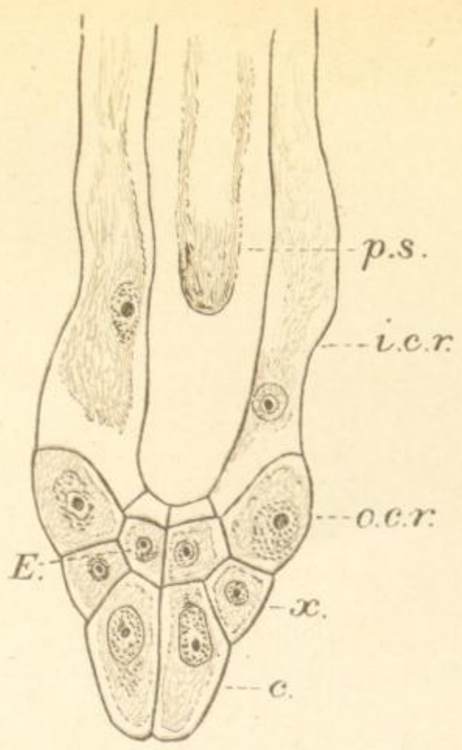

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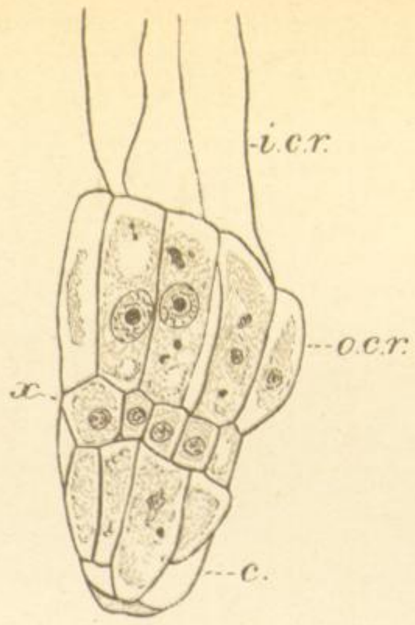

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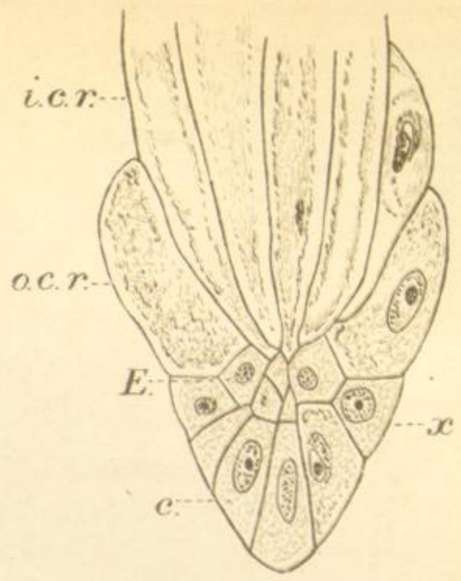

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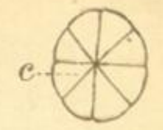

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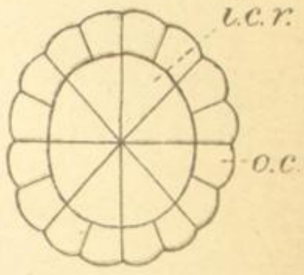
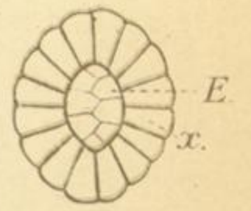


86C.



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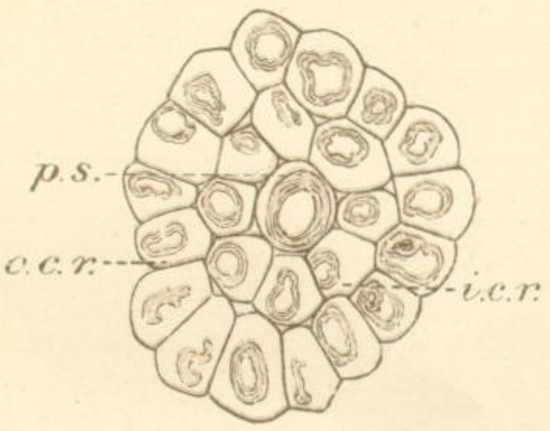

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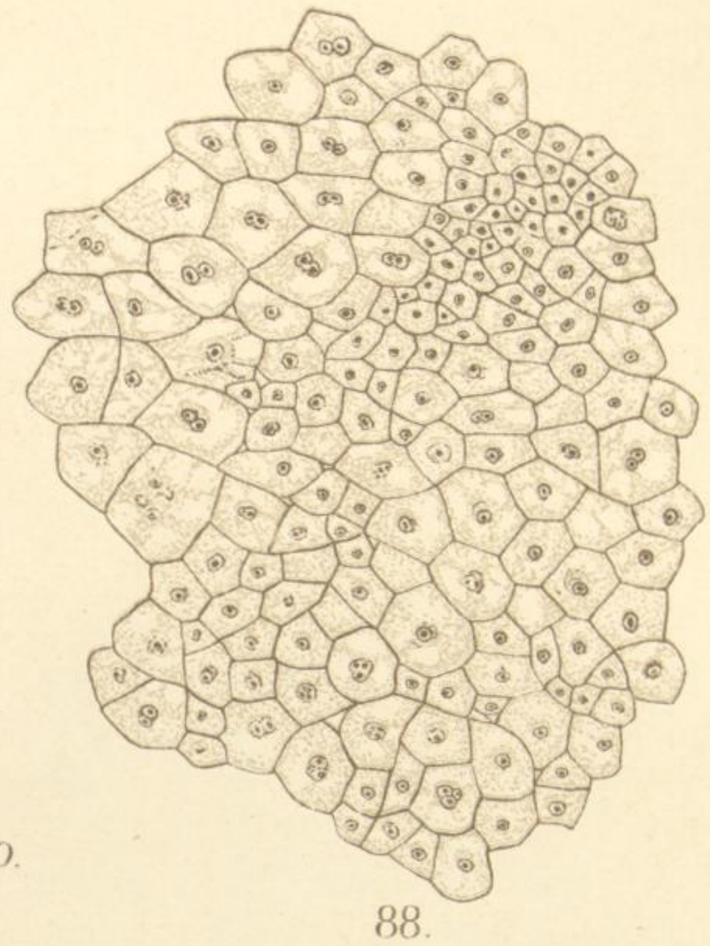

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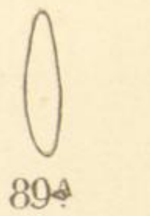

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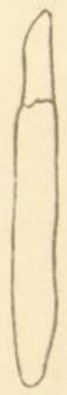
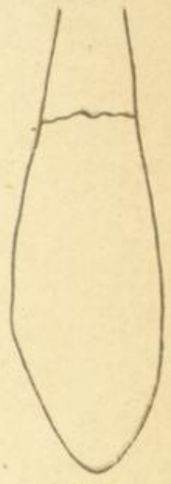


89B.

89C.

89D.



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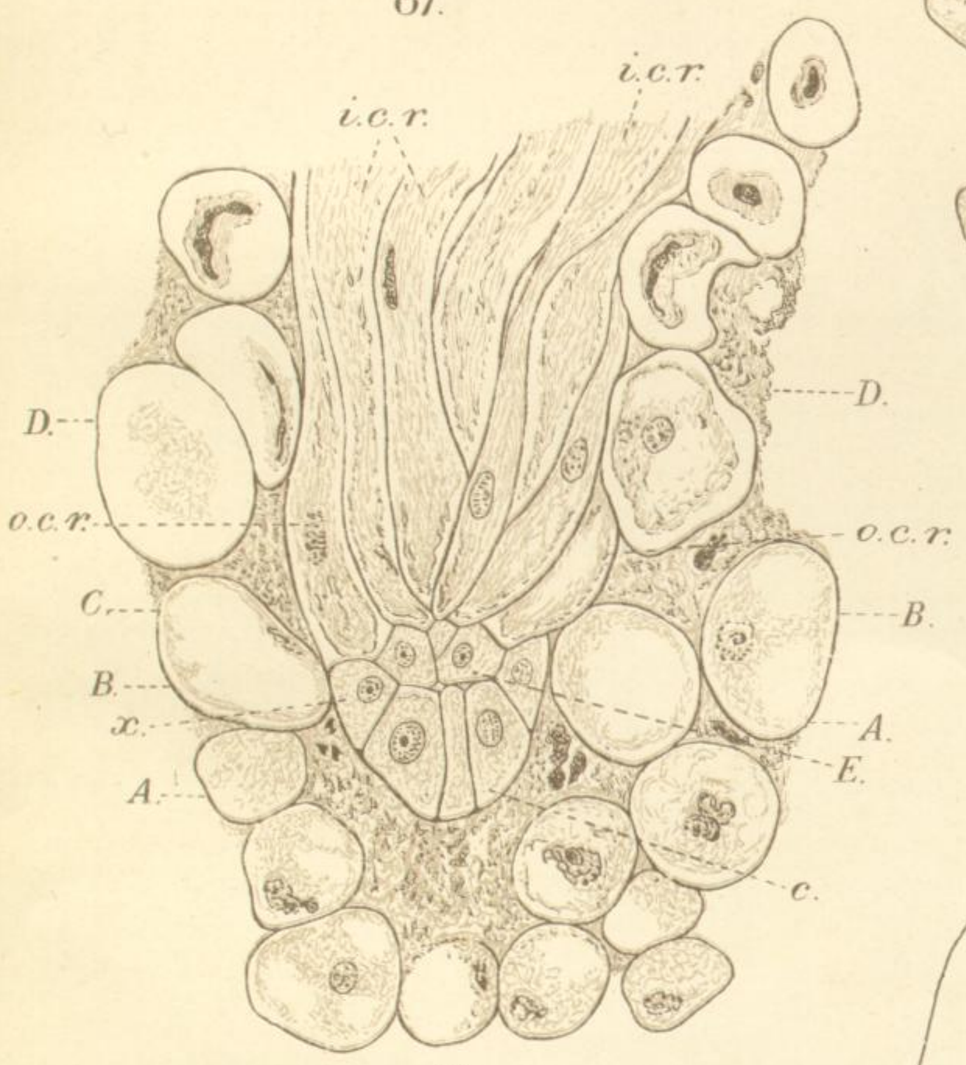

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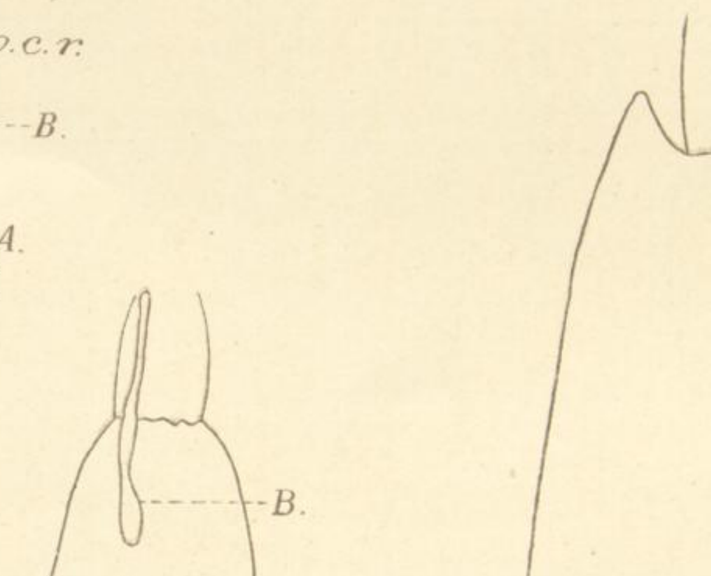

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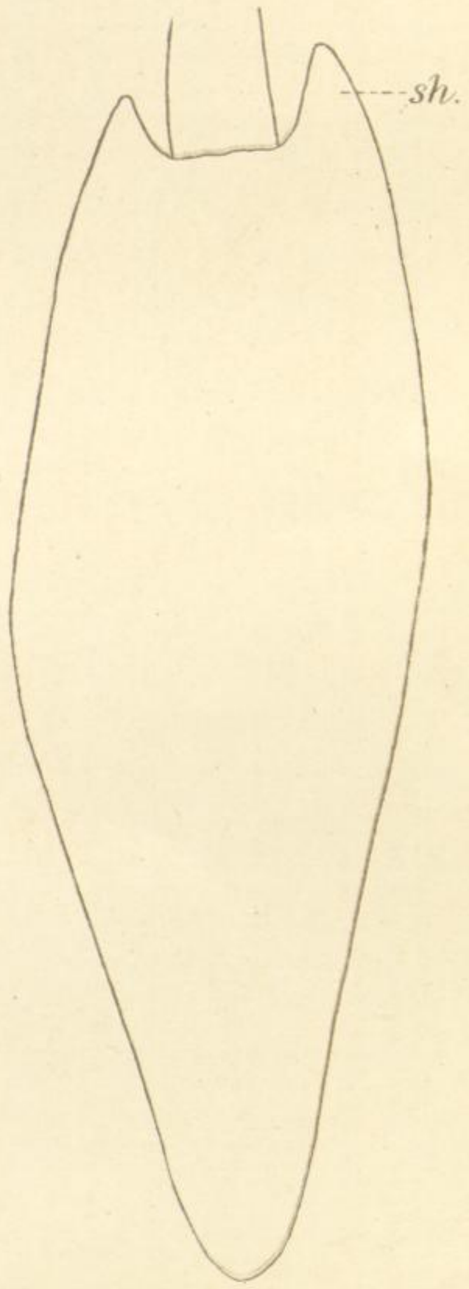

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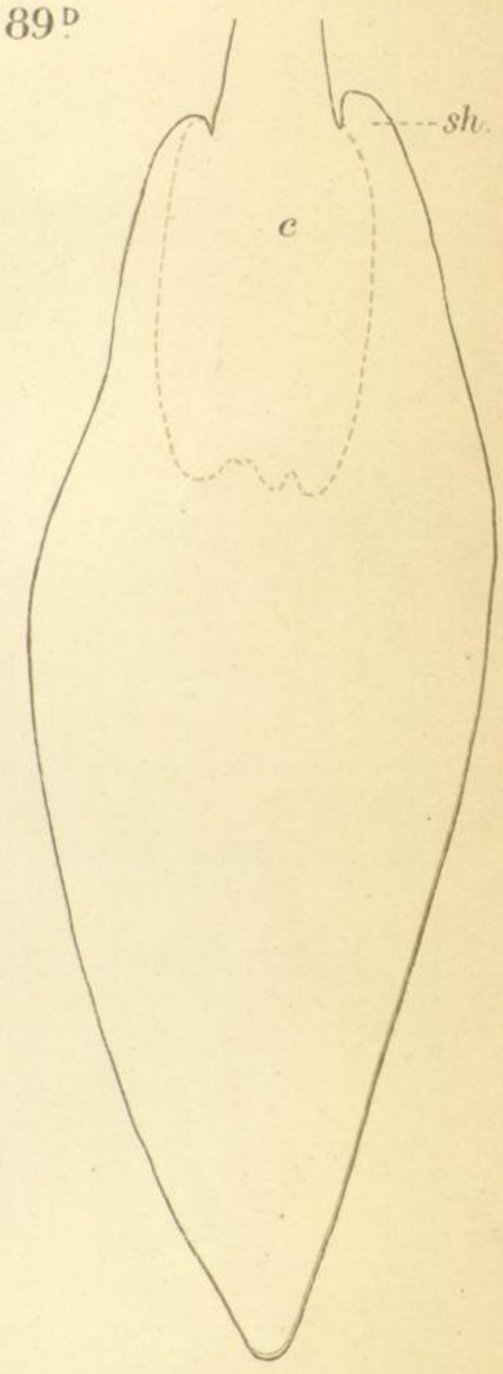

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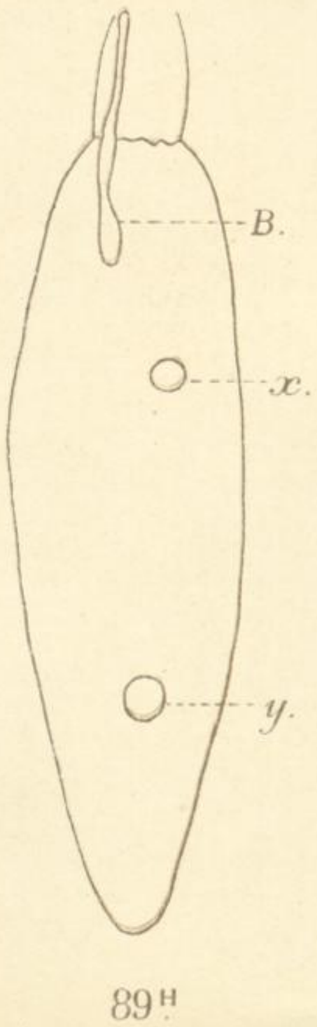
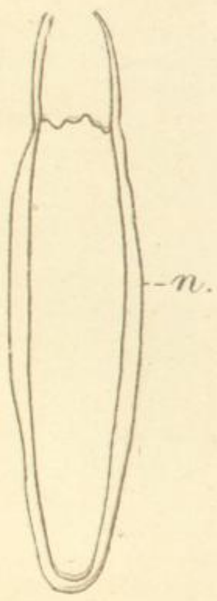


89H.



89F.

89G.